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Breeding biology of painted stork *(Mycteria leucocephala),* in an urban water body

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ABSTRACT

The Painted Stork (*Mycteria leucocephala*) is a near-threatened (NT) species within the Ciconiidae family, inhabiting the Indian Subcontinent and Southeast Asia. This nomadic Ciconiformes waterbird breeds in shallow wetland habitats from August to December in North India. From August 2023 to January 2024, we closely examined various behavior patterns exhibited by the Painted Stork during its breeding cycle. These included nest building, courtship and mating rituals, incubation, hatching, and feeding behaviors. Our research was carried out at Mataji Pond, located in Chawandiya village, Bhilwara district, Rajasthan, where we identified and observed 91 nests. These birds construct their nests on tall emergent trees, particularly *Vachellia nilotica*, strategically positioning them directly over or near water. This careful choice of nesting locations underscores their remarkable adaptability to their environment and ecological niche. We also analysed factors influencing breeding success, providing valuable insights for conservation efforts. By understanding their behavior and ecological preferences, we contribute to protecting this near-threatened species.

Figures : 17 References : 09 Table : 01
KEY WORDS : Breeding, Courtship, Feeding, Hatching, Nest building, Painted stork.

Introduction

The breeding behavior of the Painted Stork is rarely reported in Bhilwara district of Rajasthan. The Painted Stork *(Mycteria leucocephala*) is a common aquatic bird native to the Indian subcontinent, belonging to the Ciconiidae family. It is among the 19 stork species globally and is distinctive with its white plumage featuring black barring on wings and breast flight feathers[3]. Its lower back is adorned with pink tertial feathers, from which it derives its name. Both males and females exhibit similar appearances, with males generally larger. Painted stork is a piscivorous bird that requires shallow waters, around 20-25 cm deep, for foraging of fishes[8,9]. Painted Storks possess long pink legs, extended necks, and distinctive yellow, long, and decurved bills. Although resident, they locally migrate in response to environmental changes.

The IUCN classifies this species as “Near Threatened” due to concerns of rapid population decline attributed to hunting, egg collection, breeding colony disturbance, drainage, and agricultural conversion. The Ciconiidae is the only family in the order Ciconiiformes where all species of storks are classified. India hosts eight of these species, six of which, including the Painted Stork, are considered native. In Western India, known breeding colonies of the Painted Stork are mainly found in Gujarat and Rajasthan. Rajasthan is India’s largest state and its southern part is more suitable for aquatic birds due to heavy rainfall and numerous wetlands[6]. These colonial water birds prefer nesting on moderate-sized trees like *Vachellia nilotica* near water bodies. While there are reports of heronries and breeding birds in Rajasthan’s protected areas, Bhilwara district lacked any reports of Painted Stork breeding.

ACKNOWLEDGEMENTS : We are thankful to the Principal and Department of Zoology, M.L.V. Government College, Bhilwara, Rajasthan for providing the necessary facilities to carry out the study.

TABLE-1: Observations recorded during the breeding season

Parameters	31 August	30 September	31 October	30 November	31 December	31 January
No. of Painted Stork	47	89	187	49	32	11
No. of breeding pairs	22	41	78	21	06	04
No. of nests	12	23	37	12	05	02
No. of eggs	00	39	68	21	09	00
No. of hatchlings	00	15	51	67	04	00
No. of nestlings	00	00	18	89	45	00
No. of fledglings	00	00	00	126	147	24
Total number of off-springs	**297**					

This study aims to provide an analytical presentation of the breeding and ecological aspects of the Painted Stork in Bhilwara, Rajasthan (India).

Materials and Methods

We extensively observed behaviors including nest building, courtship, mating, incubation, hatching, and feeding during the period from August 2023 to January

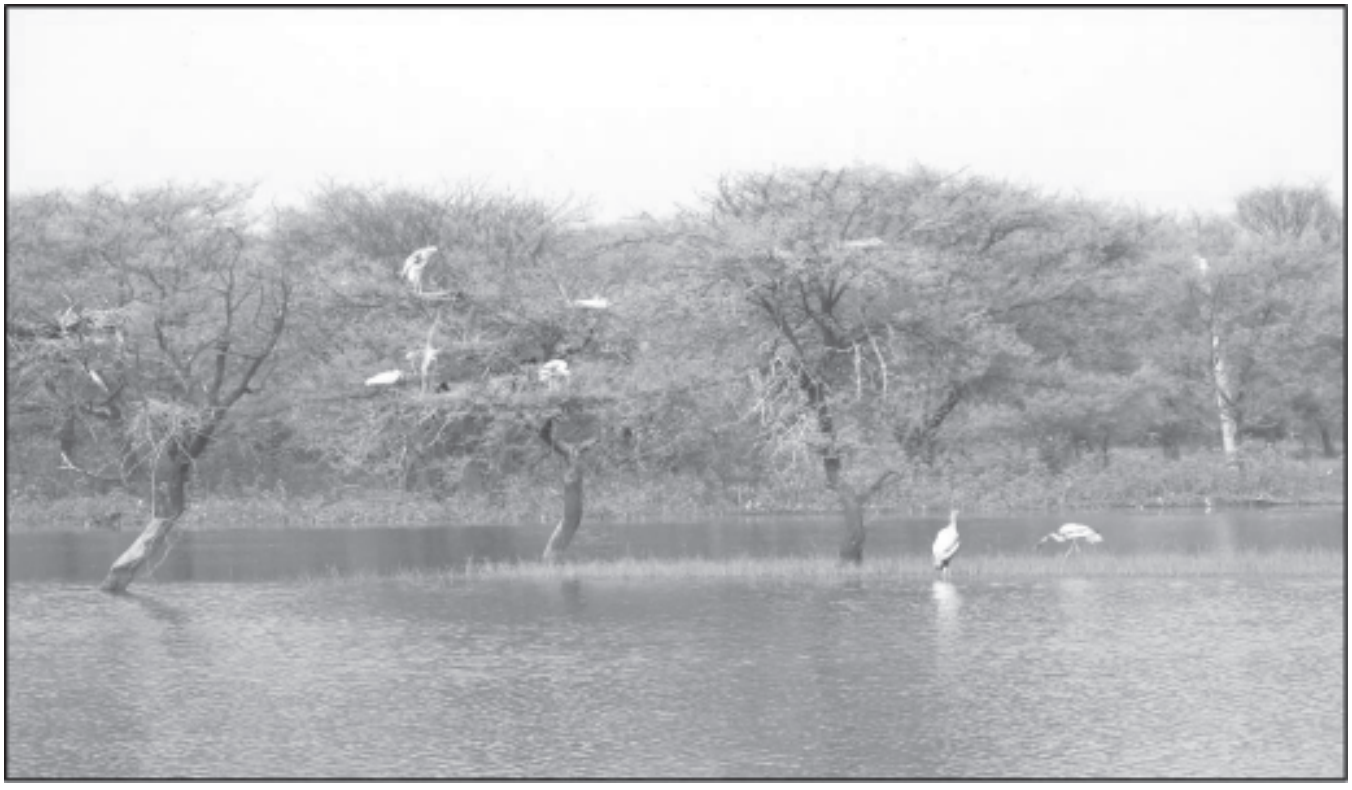

Fig. 1: Mataji Pond, Chawandiya, Bhilwara (Rajasthan, India)

Fig. 2 *Vachellia nilotica*

Fig. 3 Co-occuring bird species

Fig. 4 Nest building

Fig. 5 Collecting Stick

Fig. 6 Collecting green twig

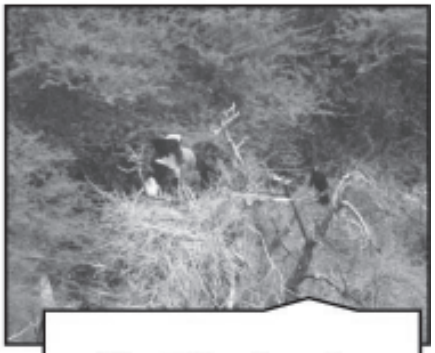

Fig. 7 Fecal matter

2024.

On March 17, 2023, Shahpura was separated from Bhilwara district and formed as a new district (Shahpura) by the state government of Rajasthan (Fig. 1). The study area selected for observing the breeding biology of the near-threatened Painted Stork was Mataji Pond in Chawandiya village (25°19’818" N 74°46’516" E) (Fig. 1) in Bhilwara district.

For nest analysis, we employed direct observation methods along with surveying nests from various nesting trees to study. The site was visited weekly from August to January to monitor the breeding behavior. We followed the guidelines related to the study of the nesting biology of birds without disturbing birds[2]. The observation was carried out using Vanguard FR-1650 binocular and the behavioral aspects were recorded using a tripod and NIKON D500 camera at different times of the day, capturing activities like nest building, courtship, mating, egg incubation, feeding, and parental care.

Observation

The presence of *Vachellia nilotica* trees (Fig. 2) in and around the waterbody makes it an ideal nesting site for Painted Storks and other avian species such as Eurasian Spoonbill *Platalea leucordia* and Little cormorant *Microcarbo niger* (Fig. 4). We observed a total of 91 nests and 415 adults during this breeding season. Painted stork had started breeding at this site in August. On the first visit, there were roughly 47 painted storks out of which 22 were the breeding pairs building 12 nests. They grew in number by September end up to 89 out of which 41 were breeding pairs. We recorded 23 nests containing 39 eggs and provided shelter to 15 hatchlings at that time. October was the peak season with the site hosting 187 painted storks of which 78 were the breeding pairs (Fig. 17). There were 51 hatchlings and 17 Nestlings visible on 31 October. The count of painted storks was reduced to 49 by the end of November. There remained 21 pairs in 12 nests along with 21 eggs. Fledglings were discovered during this time and they were 126 in number along with 67 hatchlings and 89 nestlings. There were still 5 nests on 31 December and 147 fledglings. The season ended in January and the last observation had 11 painted storks out of which 4 were breeding pairs in 2 nests and 24 fledglings. We were able to witness 297 offspring throughout the complete season. (Table-1)

Result and Discussion

Breeding phenology

Painted Storks exhibited high activity during the winter season shortly after the monsoon period because rainfall and temperature have a significant influence on the nesting activities of waterbirds[4]. We observed that

Fig. 8 Mating

Fig. 9 Egg laying

Fig. 10 Incubation

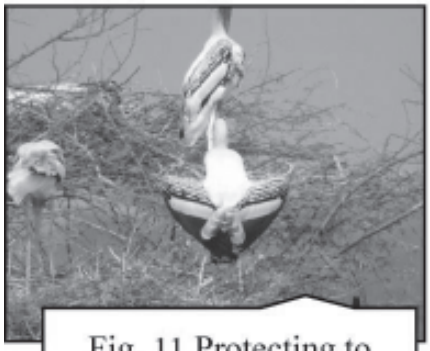

Fig. 11 Protecting to hatchlings

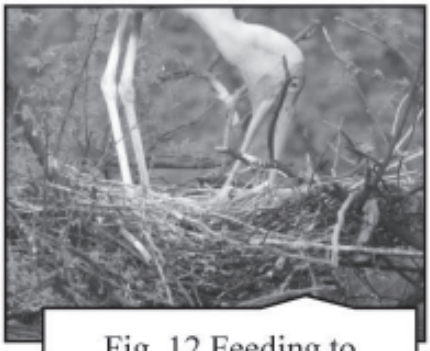

Fig. 12 Feeding to hatchlings

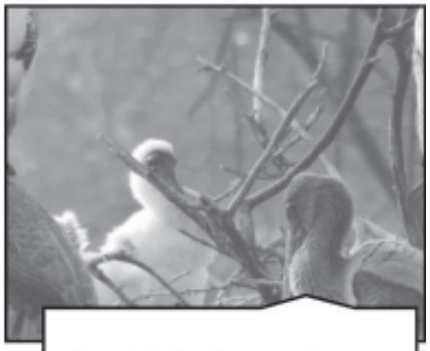

Fig. 13 Cotton ball stage

Fig. 14 Fish as a food

Fig. 15 Nestlings

Fig. 16 Fledglings

early nesters began aggregating by the end of August 2023, while late nesters appeared by the end of November 2023, aligning with the post-monsoon period. The former group served as early colonizers, whereas the latter represented late colonizers.

Courtship

Painted Storks are colonial breeders, often found in large flocks of up to two hundred individuals. They display courtship behavior involving ritual bowing which includes head-raising and lowering while performing bill-clattering[9]. After pair formation, both mates participated in nest building.

Nesting

We observed that both males and females were engaged in nest construction activities (Fig. 4). They built their nests on *Vachellia nilotica* trees submerged in the pond water. The nests were substantial platforms constructed primarily from sticks (Fig. 5) and some green leaves to protect eggs and nestlings from extreme conditions (Fig. 6). Nest building was a laborious process, with considerable time devoted to gathering nesting materials[7]. The primary considerations for selecting a nest site were the presence of ample food resources and the absence of any human disruption and interference[7]. The nest construction activity was

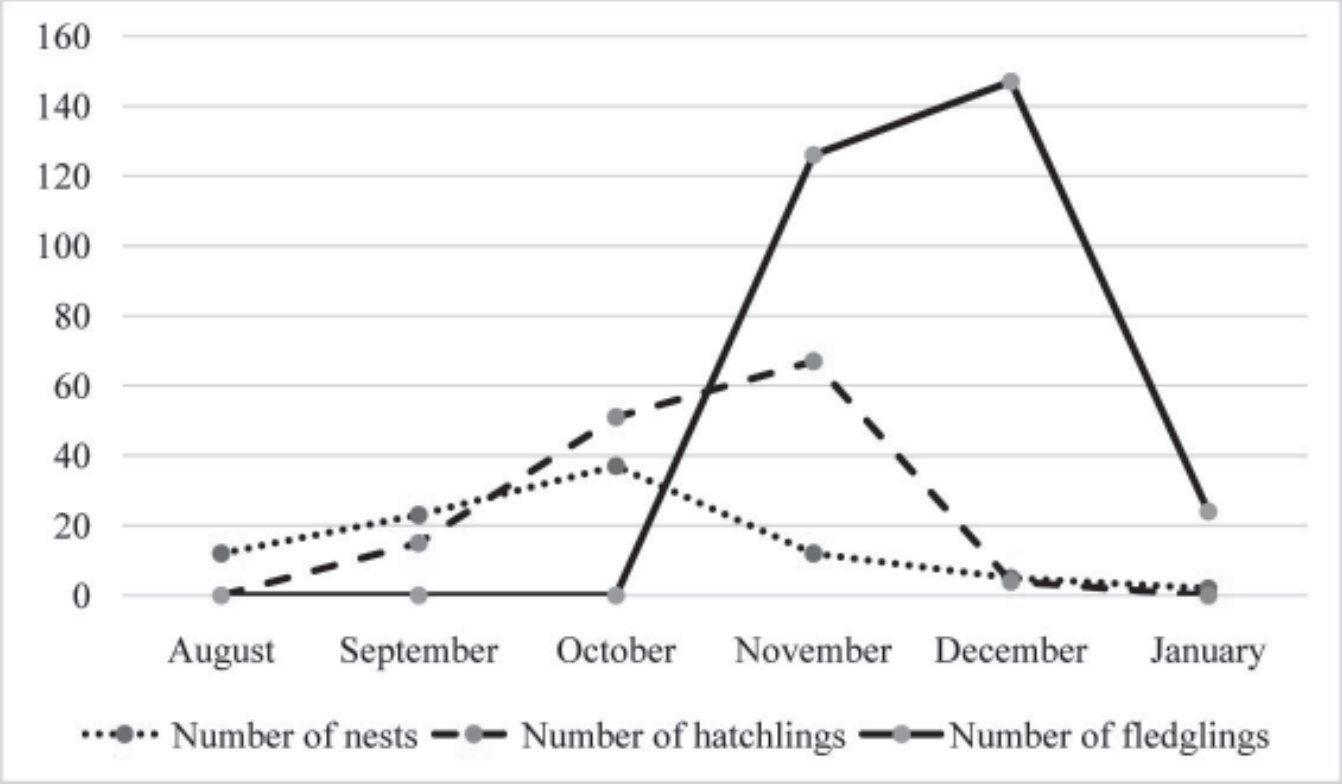

Fig. 17: Line diagram showing the number of nests, hatchlings, and fledglings

completed in the first week of October. Our observations indicated that the colony spanned a 100-meter range, with nesting trees averaging 3 to 4 meters in height. A total of 91 nests were observed on *Vachellia nilotica* trees, with some perched atop the canopy and others positioned on secondary crotches just above two feet from the water. Early arrivals occupied the canopy, while latecomers had to construct nests at lower levels. Early visitor nests were notably larger, with green foliage on nesting trees the green foliage was destroyed and turned white due to fecal matter (Fig. 7) but recovered once nests were abandoned[7].

Mating and egg-lying

After nest building and pair formation, mating (Fig. 8) and egg laying were observed in October. Painted Storks typically lay 3 to 4 eggs, with an incubation period of about 30 to 32 days (Figs. 9 & 10) and both sexes incubated eggs one after another[9]. Hatchlings were born altricial and without feathers. During mid-day heat, adults shaded the chicks by standing with wings outstretched (Fig. 11).

Feeding

Hatchlings were fed crop milk through regurgitation by both parents (Fig. 12). After 20 days from hatching, a cotton ball-like pre-nestling stage appeared which feeds only crop milk (Fig. 13). After 30 days, The prey regurgitated by parent birds in the nest was fish[5] (Fig. 14). Each parent took turns feeding the nestlings until they fledged[1], with communication evolving from loud hoarse calls to bill clattering, hissing, bowing, and wing spreading (Fig. 15). Chick fledged at 60 days. It was observed that in the last week of January, all the fledglings left their nests along with their parents (Fig. 16).

Conclusion

It can be concluded that this site is visited by the Painted storks during their breeding season. Therefore, painted storks are considered as a local migratory bird in Bhilwara district and it is one of the best breeding sites for such near-threatened avifauna. Awareness about this species should be created among local people and efforts should be undertaken for the conservation of nesting colonies of these birds. This can enhance the breeding success of the Painted Stork. Our analysis provides a foundation for conservation efforts dedicated to preserving this species in its natural habitat.

References

1. Ali S, Ripley SD. *Compact handbook of the birds of India and Pakistan together with those of Bangladesh, Nepal, Bhutan and Sri Lanka (2nd Ed)*. Oxford University Press. Oxford, UK. 1987; p737.

2. Barve S, Raman TRS, Datta A, Jathar G. Guidelines for conducting research on the nesting biology of Indian birds. *Indian BIRDS*. 2020; **16**(1) : 10-11.

3. Grimmett R, Inskipp C, Inskipp T**.** *Birds of the Indian Subcontinent (2nd Ed)*. London: Oxford University Press & Christopher Helm. 2011; p528.

4. Ismail A, Rahman F. Does Weather Play an Important Role in the Early Nesting Activity of Colonial Waterbirds? A Case Study in Putrajaya Wetlands, Malaysia. *Tropical Life Sciences Research*. 2013; **24**(1) : 1-7.

5. Kalam A, Urû AJ. Foraging behaviour and prey size of the painted stork, *Journal of Zoology*. 2008; **274** : 198-204.

6. Koli VK, Yaseen M, Bhatnagar C. Population status of Painted stork *Mycteria leucocephala* and Black-headed Ibis *Threskiornis melanocephalus* in southern Rajasthan, India. *Indian BIRDS*. 2013; **8** (2) : 39-41.

7. Kumar A, Kanaujia A. Nesting behavior of painted stork in Lucknow district of Uttar Pradesh, India. *Asian Journal of Conservation Biology*. 2015; **4**(2) : 151-153.

8. Prabhakar CS, Dudhmal D. Painted Stork (*Mycteria leucocephala*): Population status, shift in food and behavioral ecology from isolated ponds of Godavari River basin in Nanded district, India. *Science Research Reporter*. 2016; **6**(1) : 50-57.

9. Suryawanshi KR, Sundar KSG. Breeding ecology of the Painted Stork *Mycteria leucocephala* in a managed urban wetland. *Indian BIRDS*. 2019; **15**(2) : 33-37.

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Sanitation and Hygiene Condition of Urban Slums: A Study on Slums of Lucknow City, (U.P.) India

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ABSTRACT

The number of slums has significantly increased in Lucknow over the last three decades along with the expansion of cities and towns. Rapid urbanization, caused largely by a heavy influx of migrants from rural areas, has exerted severe pressure on urban housing and public services in Lucknow City. At the rate at which urban slums are increasing the infrastructure and basic services in Lucknow are not able to cope. Due to this circumstance as well as the impoverished economic circumstances of the poor migrants, a considerable number of slums have been formed where severe service deficiencies have compounded and proliferated, creating dangerous environmental conditions. In the present Study Data have been collected from primary sources, secondary sources and from focused group discussion. The study analyses the practice of sanitation and hygiene regarding water. This paper has highlighted the survey of water resource availability and quality at the source point of consumption; problems faced in getting safe drinking water; and knowledge of hygienic latrines; awareness about health. In addition to the above, the survey maintained a specific focus on, women and the young generation. The study finds that there have been some improvements in terms of knowledge of hygiene, due to the spread of awareness during corona.

Figure : 00 References : 09 Tables : 02
KEY WORDS : Hygiene; Sanitation; Slum; Water resource

Introduction

United Nations defined slums as communities characterized by insecure residential status, poor quality of housing, overcrowding, and inadequate access to safe water, sanitation, and other infrastructure[9]. With the global trend of rapid urbanization, the proliferation of informal settlements, commonly known as slums, has become a prominent feature of many cities worldwide. In the context of India, urbanization has led to the emergence and expansion of slum communities, posing significant challenges in terms of basic amenities and public health. Among these challenges, ensuring adequate sanitation and hygiene conditions within urban slums stand as a critical priority for both local governments and public health authorities. Slums are characterised by informal and unplanned settlements with poor infrastructure and inadequate sanitation unfit for human habitation[2]. Understanding the challenges faced by slum dwellers will help in formulating targeted involvement that can effectively enhance the health and well-being of slum residents while fostering sustainable urban development. In 1981, the slum population in India constituted 17.5% of the urban population which increased to 35% in 2018. In 2001, there was 23.5 per cent of households in urban areas which were living in slums. In 2011, it came down to 17.4 per cent. But there are still 13.74 million slum households and 68 million people living in the slum areas[1].In the context of Uttar Pradesh, though the state is considered one of the less urbanized states of India, it has the second-largest urban population in the country. About 22% of the population lives in urban areas in Uttar Pradesh, which constitutes more than 44 million[6]. Sanitation and health have invariably been linked together, the former being an essential condition to achieve the latter[4].Sanitation is also one of the major environmental health issues to be addressed. The Joint Monitoring Project (JMP) report of 2013 estimates that 50% of the population in India still defecates in the open. 1.9 billion people gained access to improved sanitation facilities over a period of two decades (1990 to 2011) with an average rate of 240,000 individuals gaining access every day. 8% of the Indian population is still devoid of clean water, and only 25% of the population has access to piped water on premises[9].The lack of recognized space for the urban poor in the cities' master plans has led to a significant rise in the number of slum households in the country over the decade[1].

TABLE-1 : Profile of families in the selected slums of Lucknow

Household Characteristics	Number	Percentage (%)
Family Type		
Nuclear	316	79.2
Joint	84	20.8
Number of family members		
Up to 4	280	70.1
5 - 7	40	9.9
>7	80	20.0
Religion		
Hindu	294	73.6
Muslim	105	26.4
Social Class		
OBC	101	25.2
SC/ST	212	52.9
General	87	21.9
Education		
Literate	292	73.1
Illiterate	108	26.9
Socioeconomic Status		
Upper lower	76	18.9
Lower middle	94	23.5
Lower	230	57.6

References – Personal Based Survey 2023

Access to better sanitation conditions in the form of the availability of private toilets is largely governed by the financial status of the family. An improvement in health and privacy will, therefore take place with enhanced accessibility to private toilet facilities[5]. Kaccha slums were mainly responsible for the overall burden of excreta disposal, solid waste disposal, and access to their water supply for drinking and other household purposes[3]. Improved sanitation, hygiene and water can help generate considerable national and household health savings both in terms of cost and time which in turn can be used productively. The over congested locality and house setup might be the reason for inadequate ventilation (47.3%) and lighting (25.0%).Dampness was found in about 72.4% of the surveyed households, which directly revealed the unplanned and improper construction and maintenance of the houses[7].

Study Area

The city of Lucknow is situated on the banks of river Gomti which passes through the middle of the city. The city lies at 26°30' N and 27°10 'N Latitude and 80°30' E and 81°13' E Longitude. Lucknow is the capital city of Uttar Pradesh state and one of the most prominent cities in India in terms of commerce, education, history, architecture, culture, Urdu literature *etc*.

Objectives

- To analyse the sanitation and hygiene conditions in the slums of Lucknow.
- To examine the awareness of sanitation and hygiene conditions in residents of the slums of Lucknow.

Methodology

4.1 Sources of data

In the present work, both primary and secondary data have been used. The source of primary data is a questionnaire schedule distributed among sampled respondents and through field observations. The secondary information was gathered from different sources like books, records, journals, National and International reports, reviews, websites of Government and Non-Governmental Organizations (NGOs), and reports of the Committee on Slum Statistics/Census of Lucknow city.

4.2 Sampling and collection of data

Lucknow City is divided into 6 Zones out of which 4 Zones have been selected for data collection. Data were collected from 100 households from each of the 4 selected slums. The four selected slums were Mawaiyya, Madiayon, Amausi and Chinhat Bazar.

TABLE-2 - Sanitation and Hygiene Condition

Variables	Parameters	Indicators	Mawaiyya	Madiaon	Amausi	Chinhat
X1	Main source of Drinking water	Pipe water (municipality)	31.00	41.00	36.00	49.00
		Public hand pump	69.00	59.00	64.00	51.00
X2	Quality of water consumed	Ppm 200 -400	18.00	26.00	29.00	51.00
		PPM more than 400	82.00	74.00	71.00	49.00
X3	Distance Travell to fetch clean water	1km – 2km	83	86	78	91
		More than 2km	17	14	22	9
X4	Duration for which water is available	2hours a day	27	34	33	19
		4 hours a day	61	43	57	36
		6 hours a day	12	23	10	45
X5	Drinking water handling & storage practices	Safe	25	20	22	30
		Not safe	75	80	78	70
X6	Handwashing practice before eating food	Present	78.00	76.00	72.00	82.00
		Absent	22.00	24.00	28.00	18.00
X7	Hand wash Practice before cooking food	Present	52.00	64.00	65.00	53.00
		Absent	48.00	36.00	35.00	47.00

Variables	Parameters	Indicators	Mawaiyya	Madiaon	Amausi	Chinhat
X8	Drainage for sullage	Present	38.00	11.00	9.00	95.00
		Absent	62.00	89.00	91.00	5.00
X9	Excreta disposal	Open field	78.00	67.00	72.00	66.00
		Sanitary latrine	22.00	33.00	28.00	34.00
X10	Rodent sites	Present	58.00	77.00	66.00	53.00
		Absent	42.00	23.00	34.00.	47.00
X11	Mosquito breeding sites	Present	78.00	75.00	74.00	72.00
		Absent	22.00	25.00	26.00	28.00
X12	Habit of cleanliness	Present	78.00	72.00	65.00	77.00
		Absent	22.00	28.00	35.00	23.00

References – Personal Based Survey 2023

4.3 Method and techniques of analysis

Based on the objectives and problems of the study, the data were processed and analysed through Microsoft Excel[7]. The collected data were further analysed and interpreted with the help of statistical tools like percentages and averages.

Results and Discussion

The profile of families in the selected slums of Lucknow like family type, number of family members, religion, social class, education and socioeconomic status are given (Table – 1). It shows that 79.2% of families in slums are nuclear type and only 20.8% of families are joint families. The number of family members in slums shows that 70.1 % of households have up to 4 members in the family, around 9.9% of families in slums have family members between 5 members to 7 members, and 20 % of families in slums have family members more than 7 members in the family. Religion-wise profile of respondents 73.6 % are Hindu and 26.4 % are Muslim. In the social caste group, 21.9% belong to the General class, 25.2% of respondents belong to other backward classes (OBC), and 52.9% are in Schedule Caste (SC) and Schedule tribes(ST). The Table shows the education profile of slum respondents shows that 73.1% are Literate and 26.9% are Illiterate. According to the Socioeconomic status of respondents, about 18.9% of respondents belong to the Upper lower class, 23.5% of respondents belong to the Lower middle class and 57.6% of respondents belong to the lower class.

The present study (Table-2) analysed the sanitation and hygiene condition of four selected slums of Lucknow on twelve indicators which include the main source of drinking water, quality of water consumed, distance travelled to fetch clean water, duration for which water is available, drinking water handling and storage practices, handwashing practice before cooking food, Drainage for sullage, Excreta disposal, Rodent sites, Mosquito breeding sites and habit of cleanliness.

X1-Main source of Drinking water-This indicator shows the source of water in selected slums. In Mawaiyya, 31 % of households have a piped water supply whereas 70% of households have public hand pumps in the community for water. In Madiaon, 41

households have a piped water supply, and 59 households have a public hand pump. In the Amausi slum, 65% of households have a public hand pump water supply. In Chinhat Bazar slum around 51% of households have public hand pumps installed in the community.

X2- Quality of water consumed- In Mawaiyya slum in 82% of households the quality of water is poor, and not good for drinking. In Madiaon slum 74% of households the quality of water is poor, not good for drinking. In Amausi slums in only 30 % of houses, the quality of water is fair enough. In Chinhat bazaar slum, the quality of water is fair in 51% of houses.

X3-Distance travel to fetch clean water– It is found that most of the slums don't have a direct supply of clean water and they have to travel long distances to fetch clean water. In Mawaiyya slum 83% of people have to travel for one to 2 km to fetch clean water. In Madiyaon 86% of people have to travel one to 2 km distance. In Amausi 78% of people's households have to travel one to 2 kilometres to fetch clean water. In Chinhat Bazar only 9% of households travel more than 2 kilometres to fetch clean water which shows that the Chinhat Bazar slum has better water conditions.

X4-Duration for which water is available- There have been instances in slums where water is not available for the whole day. In Mawaiyya 27 households have access to water for only 2 hours a day. In Madiaon, in 34 households water is available for 2 hours a day. In Amausi Slum 33 households have access to water for 2 hours a day. In Chinhat Bazar slum 45 households have water available for 6 hours aday.

X5-Drinking water handling and storage practices- Due to less awareness among people in slums, they do not handle drinking water safely and neither store it properly. This variable indicates the handling and storage practices in slums. In Mawaiyya, only one-fourth of households found safe handling and storage of drinking. In Madiyaon, almost 80% of households do not safely handle and store drinking water. In Amausi, only 22% of households safely handle and store drinking water. In Chinhat bazaar slum, 30% of households know about the safe handling and storage of drinking water.

X6-Hand washing practice before eating food Mawaiyya 78% of households have hand washing habits whereas 22% of people did not put much care on washing their hands before eating food. In Madiyaon, 76% of households do hand wash before eating food. In the Amausi slum, 72% of people have a hand-washing habit before eating food. In Chinhat bazar slum 82% of people have a hand-washing habit before eating food.

X7-Hand washing practice before cooking food- It is another indicator which indicates the habit of sanitation and hygiene in the slums. In Mawaiyya slum around 50% of people, take hand washing habits casually and don't wash their hands before cooking food. In Madiyaon slum 64% of people do wash their hands before cooking food. In the Amausi slum, 35% of people take it casually and do not wash their hands before cooking food.In Chinhat bazar slums 53% of people prefer to wash their hands before cooking food.

X8-Drainage for sullage- In Mawaiyya, over 38 households have drainage facilities for sullage while in 62 households drainage for sullage facilities is not present, in these households open pit is present for sullage. In Madiaon slum only in 11 households drainage for sullage is present whereas in 89 households drainage for sullage is not present. In Amausi slum mere 9 households have drainage for sullage whereas in 91 households no drainage for sullage. In Chinhat Bazaar slums have better living conditions, in this slum around 55 households have drainage for sullage whereas 45 households don't have drainage for sullage facilities.

X9- Excreta Disposal–in Mawaiyya slums around 80% of households dispose of excreta in open fields. In the Madiaon slum, 67% of househol dispose off excreta in open fields. In the Amausi slum, 72% of households dispose of excreta in open fields. In Chinhat Bazar slum 34 % of households use sanitary latrines and in this slum, it is observed that major role is played by Local NGOs in spreading awareness.

X10 – Rodents – In Mawaiyya slums around 58 households rodent sites are present. In Madiaon slums it is seen that around 77 household rodents are present, only around 23 household rodent sites are not present because of awareness of dengue disease. In the Amausi slum around 66 households rodent sites are present and around 34 households no rodents are present. In the Chinhat Bazaar slum around 53 households, no rodent sites are present because of awareness provided to them by local NGOs during corona time about cleanliness.

X11- Mosquito breeding sites – It is found that due to waterlogging in slums there are ample chances of mosquito breeding sites. In this research, it is found that, in Mawaiyya slum mosquito breeding sites are present around 78 households. In Madiaon slum around 75 households mosquito breeding sites are present. In Amausi slum around 74 houses mosquito breeding sites are present. In Chinhat Bazaar slum around 72 houses mosquito breeding sites are present.

X12 – Habit of cleanliness – This variable is added to analyse the behaviour of people in slums towards the habit of cleanliness around them. In the Mawaiya slum in 78 houses habit of cleanliness is present whereas in

22 houses habit of cleanliness is not present. In Madiaon slum in 72 houses habit of cleanliness is present. In the Amausi slum in 65 houses habit of cleanliness is present whereas in 35 houses habit of cleanliness is not present. In Chinhat bazar slum, in 77 houses habit of cleanliness is present only 23 houses do not have a habit of cleanliness.

Conclusion

The present study showed that sanitary and hygiene conditions are very poor in slums. The behaviour of people in slums towards sanitation and hygiene is negligible they completely rely on the support of others. Government support is needed to bring change in the sanitation and hygiene conditions of slums in Lucknow. It has been observed that role of NGOs played a major role in spreading awareness of sanitation and hygiene.In Chinhat Bazar slum the sanitation and hygiene conditions are somehow better because the people of slum are proactive towards sanitation and hygiene. In Mawaiyya slum hygiene conditions are very poor because of less awareness of cleanliness.In Madiaon and Amausi it is observed that clean drinking water and water availability duration is poor, people of slum are struggling to meet their daily basic needs.

References

1. *Census of India | Office of the Registrar General & Census Commissioner, India* (Issue March). (2011). http://censusindia.gov.in/2011-prov-results/data_files/india/pov_ popu_total_presentation_2011. pdf
2. Davis M. Planet of Slums. In *New York, Verso.* 2008; **49**(3) : https://doi.org/10.1177/0306396807085905
3. Gupta A, Sengar M, Manar M, Bansal U, Singh SK. Tracking Water, Sanitation, and Hygiene Practices: Waste Management and Environmental Cleaning in the Slums of North India. *Cureus*. 2023; **15**(7) : https://doi.org/10.7759/cureus.42067.
4. Mara D, Lane J, Scott B, Trouba D. Sanitation and Health. *PLoS Medicine*. 2010; **7**(11) : https://doi.org/10.1371/journal.pmed.1000363.
5. Nagpal A, Hassan M, Siddiqui MA, Tajdar A, Hashim M, Singh A, Gaur S. Missing basics: a study on sanitation and women's health in urban slums in Lucknow, India. *GeoJournal*. 2021; **86**(2) : 649–661. https://doi.org/10.1007/s10708-019-10088-0.
6. Regional Centre for Urban & Environmental Studies. *Slum Free City Plan of Action - Lucknow*. 2012; 119. http://mohua.gov.in/upload/uploadfiles/files/23UP_Agra_sfcp-min.pdf
7. Shukla M, Agarwal M, Rehman H, Yadav K, Imchen T. Housing and sanitary conditions in slums of Lucknow, capital of Uttar Pradesh. *International Journal of Medical Science and Public Health*. 2016; **5**(6) : 1153. https://doi.org/10.5455/ijmsph.2016.30092015171.
8. UNHSP. *The Challenge of Slums: Global Report on Human Settlements 2003*. UN-Habitat. 2012; https://doi.org/10.4324/9781849772907.
9. WHO/ UNICEF. *Joint Monitoring Program (JMP) for Water Supply, Sanitation and Hygiene Report: Progress on household drinking water, sanitation and hygiene 2000*. http://apps.who.int/bookorders.

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A phytochemicals based formulation induced faster wound healing of excision wounds in *wistar* rats

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ABSTRACT

The present study involved development and scientific validation of wound healing potential of three phytochemical-based formulations at different concentration in experimental rat model. Formulation-I consisted of ethanolic extracts of *T. erecta*, *T. procumbens*, *A. vera* and *C. longa*. Formulation-II consisted of ethanolic extracts of *A. indica, F. benghalensis*, *A. vera* and *C. longa*. Formulation-III contained ingredients of both Formulations-I and II. The extracts were analyzed quantitatively, revealing that *T. erecta* had the highest content of total phenols (41.61±0.10 mg GAE/g) and flavonoids (52.50±0.53 mg QE/g) and tannins were found in *T. procumbens* (59.61±0.15 mg TC/g). Wound healing functions of formulations were assessed by topical application on excision wounds made in *Wistar* rats. The healing process was monitored by measuring the degree of wound contraction on alternate days. The polyherbal Formulation-III (5% concentration) was found to achieve complete wound contraction (Significant pd"0.0001) on 18th day in comparison to other Formulations. The study demonstrated that the Formulation-III was found to have the most potential in achieving rapid wound healing rate.

Figures : 03 References : 33 Tables : 05
KEY WORDS : Excision wound, Phytochemicals, Polyherbal formulation, Wound healing.

Introduction

Wounds are physical skin injuries that result in an opening or break of the skin that disrupts structural and functional integrities of the skin tissues. These alterations may or may not be associated with the loss or damage of underlying connective tissues (bone, cartilage, fat, blood and lymphatic tissue). Wound healing is the process involving four overlapping phases, *viz.,* hemostasis, inflammation, proliferation and remodeling[4,6,14]. Speedy and rapid wound healing is necessary for the survival and protection against the edema and skin ulcers which may prove lethal to the organism on no treatment[18].

The *Tagetes erecta*, *Tridax procumbens*, *Azadirachta indica, Ficus benghalensis*, *Curcuma longa* and *Aloe vera* exhibit a wide spectrum of biological activities and are used as traditional medicines for household remedies against various human ailments in Ayurveda[20]. *T. erecta* also known as Marigold (Family-Asteraceae)[32] and has anti-microbial, anti-inflammatory, hepatoprotective, anti-parasitic, anti-septic, anti-oxidant, analgesic and wound healing properties[2,9]. Studies have

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TABLE-1: Herbal components of F-I, F-II and F-III

F-I	F- II	F-III
T. erecta	-	*T. erecta*
T. procumbens	-	*T. procumbens*
-	*A. indica*	*A. indica*
-	*F. benghalensis*	*F. benghalensis*
A. vera	*A. vera*	*A. vera*
C. longa	*C. longa*	*C. longa*

shown that it can increase platelet and white blood cell count and reduce bleeding and clotting times[16]. Topical application of *T. erecta* flower paste has resulted in faster wound healing in animals and extracts have also exhibited oral anti-ulcer activity[7]. The *T. procumbens* (Ghamara) belonging to the Asteraceae family has been extensively used in the Ayurvedic system of medicine for treatment of cuts, wounds and burns[33] due to the presence of pharmacological activities like anti-inflammatory, anti-bacterial and anti-oxidant potentials[8]. The herb contains bioactive components belonging to alkaloids, phenols, flavonoids, carotenoids, B sitosterol, fumaric acid, luteolin, quercetin, tannin, *etc.*[30]. Neem, scientifically known as *A. indica* (Family- Meliaceae), contains bioactive compounds in its every part like seeds, leaves, roots, bark and trunk. *A. indica* possesses anti-inflammatory, anti-fungal, and anti-bacterial properties that aid in wound healing. It also contains amino acids, vitamins, and main active ingredients such as nimbidin, nimbin and nimbidol that play important role in wound healing processes particularly in the proliferation phase, formation of collagen and angiogenesis[14,29]. The *F. benghalensis* (Family-Moraceae), also known as the Indian Banyan tree, has been used in traditional medicine to treat conditions such as dysentery, diabetes and nervous disorders. Its parts have antimicrobial, antioxidant, anti-inflammatory, anti-ulcer, and wound healing properties[15]. A study reported that ethanolic and aqueous extracts of *F. benghalensis* leaves demonstrated a decrease in epithelization and an increase in the rate of wound contraction in an excision wound model[10]. The *A. vera* plant from the Liliaceae family has been traditionally used to treat burns, allergic reactions, arthritis, indigestion, ulcers, diabetes, skin diseases, and digestive system inflammation[1]. *A. vera* extract has anti-inflammatory properties, promote collagen synthesis, skin regeneration and blood supply essential for wound healing[12]. It contains bioactive compounds such as flavonoids, alkaloids, tannins, terpenoids, polyphenols, amino acids and vitamins and reduces wound-related bacterial infections[11]. The *C. longa* (family- Zingiberaceae) is well known for its anti-microbial functions[22]. Curcumin has a wide range of biological effects including anti-inflammatory, anti-oxidant, anti-tumor, anti-bacterial and anti-viral activities[21]. Curcuminoids, the active ingredients of *C. longa* are known to be beneficial in treatment of skin diseases and enhance the wound healing and skin regeneration[28].

Several other herbs/plants have been reported to play a crucial role in wound healing process[26]. Polyherbal formulations not only accelerate wound healing process with minimum or no side effects *i.e.,* they are safe, non-toxic and can be administered over longer periods[25]. Hence the polyherbal formulations would have longer acceptability in treatment of wounds with high efficacy. More than 70% of wound healing pharma products are of plant origin[24]. The present study demonstrated superior wound healing potentials of different polyherbal formulations on excision wounds made on Wistar rats.

Materials and Methods

Collection and identification of plant materials

Mature *A. indica*-leaves, *T. procumbens*-whole plant*, F. benghalensis*-bark, and *A. vera*-leaves were collected from 'Charak Udhyan' (Medicinal plants garden) of Jiwaji University, Gwalior. The used

TABLE-2 : Quantitative analysis of phytochemical extracts

Extracts	Total Phenols (mg GAE /g)	Total Flavonoids (mg QE / g)	Total Tannins (mg TC / g)
T. erecta	41.61±0.10	52.50±0.53	9.920±0.24
T. procumbens	17.14±0.23	34.00±0.52	59.61±0.15
A. indica	15.15±0.53	9.420±0.60	14.17±0.60
F. benghalensis	12.23±0.90	8.970±1.12	44.01±0.41
A. vera	34.28±0.36	40.27±1.00	19.51±0.32

Results are expressed in Mean ± SEM (n=3)

T. erecta-flowers were collected from the temple of the Jiwaji University campus. The plant specimens were authenticated by the Institute of Ethnobiology, Jiwaji University. The dried plants/plant parts were mounted as herbarium specimens in the Institute and have been assigned code names *viz.*, *T. erecta* (IOE-438), *A. indica* (IOE-439), *T. procumbens* (IOE-440), *F. benghalensis*, (IOE-435) and *A. vera* (IOE-437). Curcumin purchased from Pukhraj herbals, Mandsaur India and it was used directly.

Preparation of extracts

The selected parts of plants/herbs (*T. erecta*-flowers, *T. procumbens*-whole plant, *A. indica*-leaves and *F. benghalensis*-bark) were washed. Materials were dried at room temperature in shade for 7 to 10 days then grounded to powder separately using a mechanical grinder. Crude powder was Soxhlet extracted separately using 95% ethanol. The extract was dried up at *45ºC in a hot air oven for 2-3 days. F*resh *A. vera* leaves gel was ground to homogenous gel and left for 24 hours on a magnetic stirrer adding 95% ethanol for uniform mixing. The solution was centrifuged at 10,000 rpm for 30 min., supernatant was dried under the hot air oven at *45ºC* for 2-3 days, and the powder was collected and stored at 4°C in an airtight bottle.

Characterization

Quantitative analyses

The total phenolic constituents of various extracts were determined by *Folin-Ciocalteu* method[27] using Gallic acid as standard and results are expressed as mg of gallic acid equivalents per g (mg GAE/g) of extract. The flavonoid constituents were determined by colorimetric assay[27] using Quercetin as standard and the results are expressed as mg QE/g of extract. The tannins were determined by the *Folin-Ciocalteu* method[3]. Tannic acid (100 to 1000µg/ml) was used as a standard. The tannin content was expressed as mg of TA/g of extract.

Preparation of polyherbal ointment/formulation

The base was prepared by melting cetyl alcohol and soft wax paraffin, followed by the addition of liquid paraffin. Each polyherbal formulation (I, II, and III) was created by mixing specific amounts of ingredients/ extracts (refer to Table 1) and then homogenized to form a smooth ointment suitable for topical application on wounds. Four different concentrations (2%, 5%, 10%, and 25% w/w) of each formulation (F-I, F-II and F-III) were made and stored at room temperature in plastic containers.

Experimental rodents

Wistar rats weighting about 200-250g obtained from Animal facility of the Defence Research Development Establishment (DRDE), Gwalior were acclimatized at 25°C±2°C and with a humidity of 50% - 60% for two weeks' time before making excision wounds. The animals were fed on standard pellet diet and provided water *ad libitum*.

Creation of excision wounds on *Wistar* rats

Rats were anesthetized with diethyl ether, hair of the dorsal thoracic region was shaved off with an electrical shaver and disinfected with 70% alcohol and a skin area of 2 X 2 cm^2 (~400 mm^2 diameter circular

area) on the dorsal thoracic region was excised with surgical scissor[33]. The day of wound creation was considered a zero-day. The formulation was topically applied evenly on the wound area, once a day till complete healing of the wound achieved.

Experiment- I: A total of six groups (6 rats in each group) were made as detailed below-

Group 1- Normal Control (Base)

Group 2- Reference (Betadine)

Group 3- Formulation-I (2%)

Group 4- Formulation-I (5%)

Group 5- Formulation-I (10%)

Group 6- Formulation-I (25%)

Experiment- II: A total of six groups (6 Rats in each group) were made as detailed below-

Group 1- Normal Control (Base)

Group 2- Reference (Betadine)

Group 3- Formulation-II (2%)

Group 4- Formulation-II (5%)

Group 5- Formulation-II (10%)

Group 6- Formulation-II (25%)

Experiment- III: A total of six groups (6 Rats in each group) were made as given detailed below-

Group 1- Normal Control (Base)

Group 2- Reference (Betadine)

Group 3- Formulation-III (2%)

Group 4- Formulation-III (5%)

Group 5- Formulation-III (10%)

Group 6- Formulation-III (25%)

Assessment of wound area contraction

Wound area was marked by tracing the raw wound area on transparent paper with a permanent marker and the area was measured on graph paper. The wound area was measured at 4 day intervals till complete healing was achieved following topical application of a given formulation. Photographic image of wound area from a fixed distance of 15 cm was taken and documented. Percentage of wound contraction was calculated by using the formula given below[33].

$$\text{Wound contraction \%} = \frac{[\text{Initial wound area} - \text{Wound area following treatment}]}{\text{Initial wound area}} \times 100$$

The study protocol was approved by the Institutional Animal Ethics Committee of Jiwaji University (Number- IAEC/JU/21).

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 5.0 software (GraphPad Prism software Inc., La Jolla, Ca). The results were analyzed using one-way analysis of variance (ANOVA). P values *pd"0.05, **p<0.01, ****p<0.001, ****p<0.0001. All values are presented as mean ± the standard error of the mean (SEM).

Results

Quantitative analyses

Quantitative estimation of total phenols, flavonoids and tannin were done on ethanolic extracts of all phytochemical ingredients and results are shown in the Table 2. The highest content of total phenols (41.61±0.10 mg GAE/g) and flavonoids (52.50±0.53 mg QE/g) were found in *T. erecta*. Maximum amount of tannins were recorded from *T. procumbens* (59.61±0.15 mg TC/g), followed by *F. benghalensis* (44.01±0.41 mg TC/g), *A. vera* (19.51±0.32 mg TC/g), *A. indica* (14.17±0.60 mg TC/g) and *T. erecta* (9.920±0.24 mg TC/g).

Wound healing potentials of different phytochemical formulations

Wound contractions recorded on topical application with different concentrations *viz.,* 2%, 5%, 10% and 25% of F-I as measured on day 20th were 85.35%, 100%, 94.04% and 88.50% respectively. F-I 5% on topical application showed the maximum wound contraction (100%) which is significant (pd"0.001) as compared to the reference (Table-3 and Fig. 1).

Wound contractions recorded on topical application with different concentrations of F-II *viz.*, 2%, 5%, 10% and 25% measured on day 22nd were 88.75%, 100%, 98.02% and 82.12% respectively. F-II 5% on topical application showed the maximum wound contraction (100%) which is significant (pd"0.01) as compared to reference (Table-4 and Fig. 2).

Wound contractions recorded on topical application with different concentrations of F-III *viz.*, 2%, 5%, 10% and 25% on day 18th were 91.03%, 100%, 97.37%, and 87.50% respectively. F-III at 5% on topical application showed the maximum wound contraction (100%) which is highly significant (pd"0.0001). 5% of F-III showed superior wound contraction from 4th day onwards and angiogenesis was significantly higher than

TABLE-3: Wound healing potential of F-I on excision wounds in *Wistar* rat model

Experimental Groups	Wound area (mm^2)					
	0^{th} day	4^{th} day	8^{th} day	12^{th} day	16^{th} day	20^{th} day
Group 1 (Control)	400.0±2.2 (0.0%)	386.7±6.6 (3.33%)	336.5±22.0 (12.98%)	256.2±6.6 (35.95%)	190.0±5.7 (52.50%)	121.5±6.6 (69.63%)
Group 2 (Reference)	398.0±4.2 (0.0%)	373.3±6.6 (6.21%)	336.7±12.0 (15.40%)	246.7±20.2 (38.02%)	127.1±13.2 (68.09%)	62.3±5.6*a (84.35%)
Group 3 F- I (2%)	400.0±5.6 (0.0%)	370.0±11.5 (7.50%)	333.2±14.5 (16.70%)	273.5±8.5 (31.63%)	130.3±102 (67.43%)	59.2±8.2*a (85.20%)
Group 4 F- I (5%)	400.0±4.5 (0.0%)	321.2±8.1 (19.70%)	246.7±4.6 (38.33%)	113.2±10.4 (71.70%)	23.5±15.3 (94.13%)	0.0±0.0***a*b (100%)
Group 5 F- I (10%)	396.0±8.9 (0.0%)	350.0±5.1 (11.62%)	296.7±9.3 (25.08%)	198.4±10.5 (49.90%)	89.2±11.5 (77.47%)	23.6±8.3**a (94.04%)
Group 6 F- I (25%)	398.0±4.6 (0.0%)	370.2±5.3 (6.98%)	315.0±4.2 (20.68%)	234.0±10.5 (41.2%)	121.3±4.8 (69.52%)	45.5±7.2**a (88.50%)

Data are expressed as mean ± SEM. Wound area is expressed in $mm^{2.}$ Figures in parentheses indicate percentage of wound area and is taken as measure of wound healing * $p<0.05$, ** $p<0.01$, *** $p<0.001$. Data were analyzed by One way ANOVA, **a** = compared with control , **b** = compared with the reference.

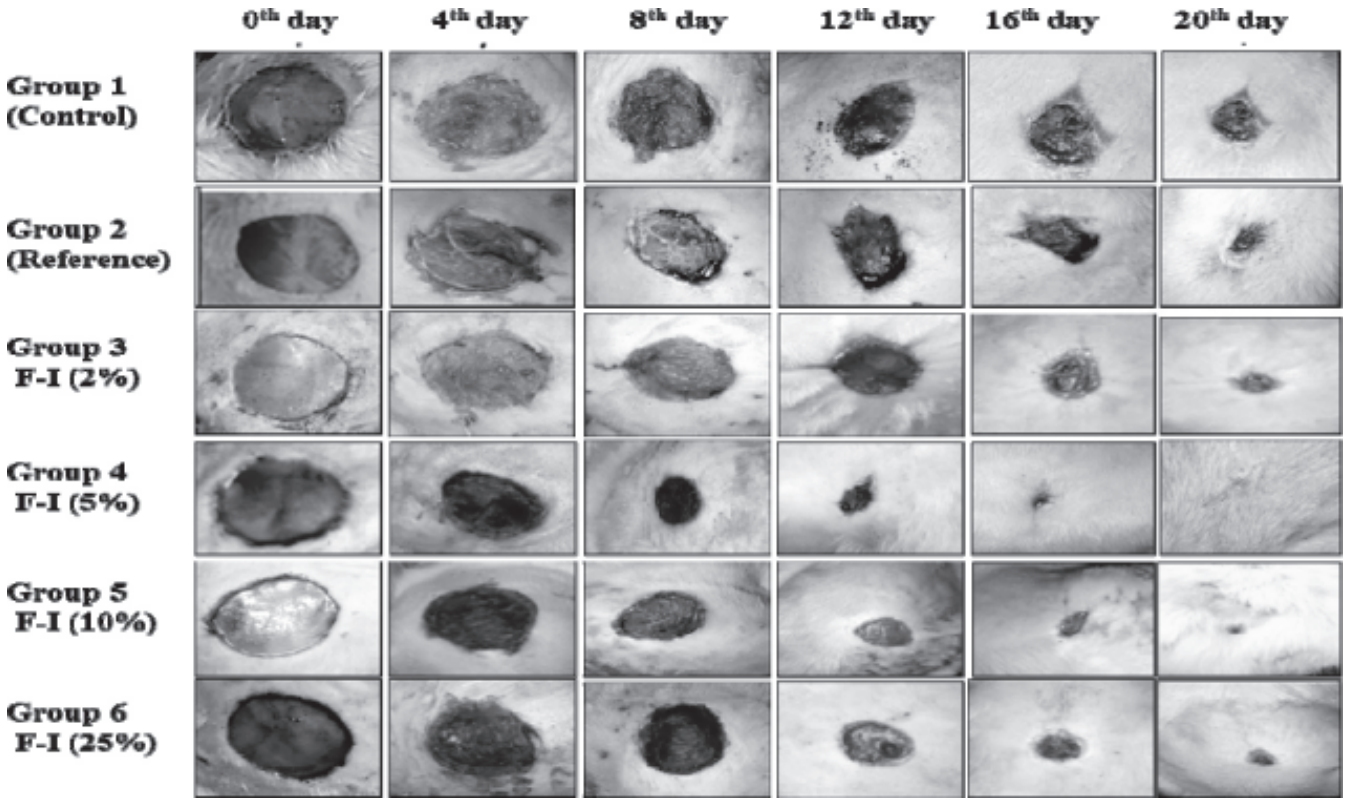

Fig. 1: Images of wound areas in different groups of experiment I at different time intervals

TABLE-4: Wound healing potential of F-II on excision wounds in *Wistar* rats

Experimental Groups	Wound area (mm^2)						
	0th day	4th day	8th day	12th day	16th day	20th day	22nd day
Group 1 (Control)	400.0±2.2 (0.0%)	386.7±6.6 (3.33%)	336.5±22.0 (12.98%)	256.2±6.6 (35.95%)	190.0±5.7 (52.50%)	121.5±6.6 (69.63%)	76.5±4.2 (80.55%)
Group 2 (Reference)	398.0±4.2 (0.0%)	373.3±6.6 (6.21%)	336.7±12.0 (15.40%)	246.7±20.2 (38.02%)	127.1±13.2 (68.09%)	62.3±5.6 (84.35%)	10.8±4.8 (97.11%)
Group 3 F- II (2%)	401.0±2.2 (0.0%)	360.0±1.2 (10.22%)	315.3±10.3 (21.37%)	247.2±12.4 (38.35%)	163.3±6.4 (59.28%)	89.3±9.7 (77.73%)	40.5±10.2*a (88.75%)
Group 4 F- II (5%)	400.8±6.3 (0.0%)	343.3±8.4 (14.35%)	276.4±9.3 (31.04%)	196.0±10.5 (51.10%)	62.7±11.8 (84.36%)	13.1±4.3 (96.73%)	0.0±0.0**ab (100%)
Group 5 F- II (10%)	399.0±5.3 (0.0%)	366.7±5.8 (8.10%)	318.0±4.5 (20.30%)	266.3±11.5 (33.26%)	196.3±11.0 (50.73%)	66.5±14.2 (83.33%)	7.9±5.6*a (98.02%)
Group 6 F- II (25%)	400.0±8.5 (0.0%)	386.7±6.5 (3.33%)	336.5±4.8 (15.88%)	293.2±6.5 (26.7%)	240.0±4.3 (40.0%)	153.3±6.2 (61.68%)	73.0±7.1*a (82.12%)

Data are expressed as mean ± SEM. Wound area is expressed in mm^2. Figures in parentheses indicate % of wound area and is taken as measure of wound healing * $p<0.05$, ** $p<0.01$. Data were analyzed by One way ANOVA, **a** = compared with control, **b** = compared with the reference.

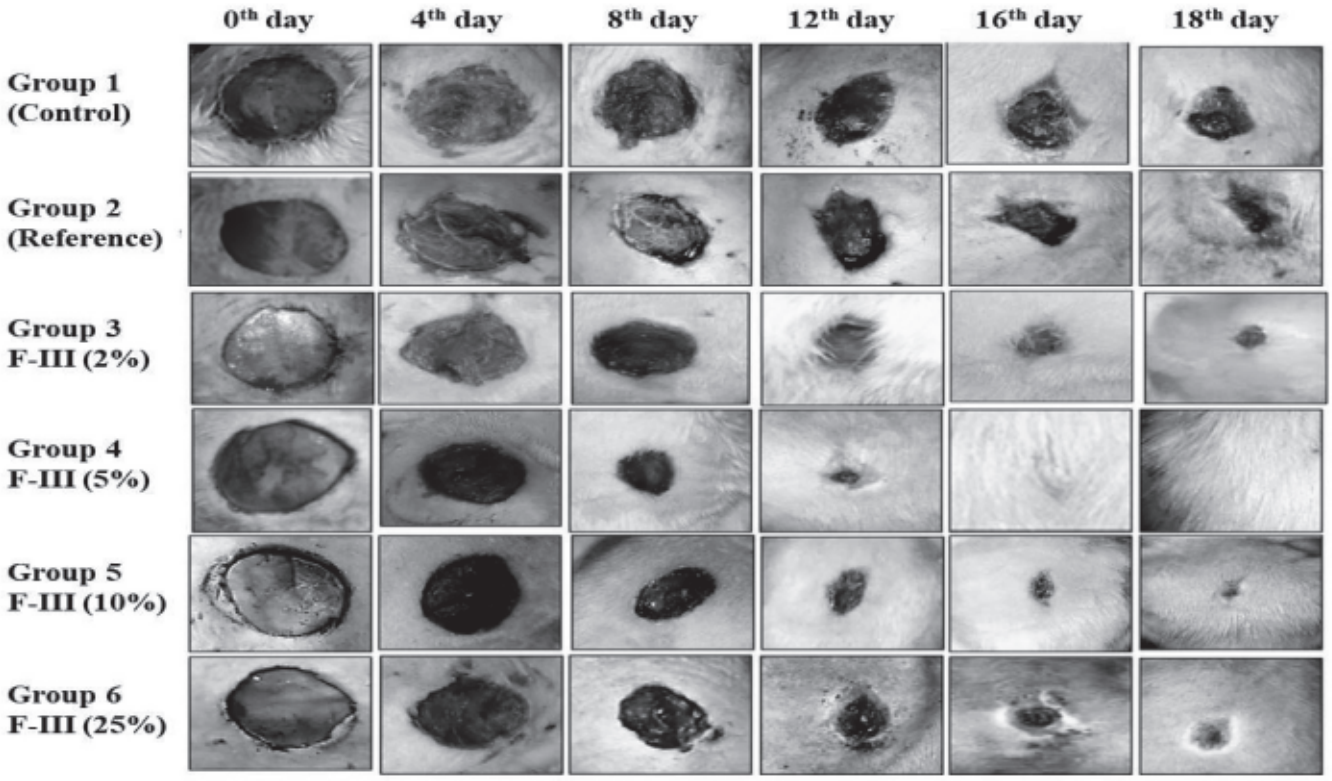

Fig. 2: Images of wound areas in different groups of experiment II at different time intervals

TABLE-5: Wound healing potential of Formulation III on excision wound in *Wistar* rats

Experimental Groups	Wound area (mm^2)					
	0^{th} day	**4^{th} day**	**8^{th} day**	**12^{th} day**	**16^{th} day**	**18^{th} day**
group 1 (Control)	400.0±2.2 (0.0%)	386.7±6.6 (3.33%)	336.5±22.0 (12.98%)	256.2±6.6 (35.95%)	190.0±5.7 (52.50%)	157.5±6.6 (60.63%)
Group 2 (reference)	398.0±4.2 (0.0%)	373.3±6.6 (6.21%)	336.7±12.0 (15.40%)	246.7±20.2 (38.02%)	127.1±13.2 (68.09%)	82.3±6.6*a (79.32%)
Group 3 F-III (2%)	395.6±3.5 (0.0%)	355.3±6.6 (10.26%)	280.4±11.5 (29.17%)	190.0±10.0 (52.01%)	73.3±13.2 (81.49%)	35.5±6.6**ab (91.03%)
Group 4 F- III (5%)	402.0±6.0 (0.0%)	310.0±11.5 (22.90%)	206.7±8.8 (48.59%)	70.0±11.5 (82.59%)	2.5±11.8 (99.38%)	0.0±0.0****ab (100%)
Group 5 F- III (10%)	398.9±8.5 (0.0%)	340.0±5.7 (14.77%)	280.0±8.8 (30.81%)	151.3±12.0 (62.07%)	37.8±15.2 (90.52%)	10.5±6.6***a**b (97.37%)
Group 6 F- III (25%)	396.0±7.2 (0.0%)	346.7±6.6 (12.47%)	273.0±5.7 (31.08%)	198.0±5.7 (52.0%)	98.8±4 (75.18%)	49.5±6.6**a*b (87.50%)

Data are expressed as mean ± SEM. Wound area is expressed in mm^2. Figures in parentheses indicate % of wound area and is taken as measure of wound healing *p<0.05, **p<0.01, *** p<0.001, ****p<0.0001. Data analyzed by One way ANOVA, **a** = compared with control (Base), **b** = compared with the reference.

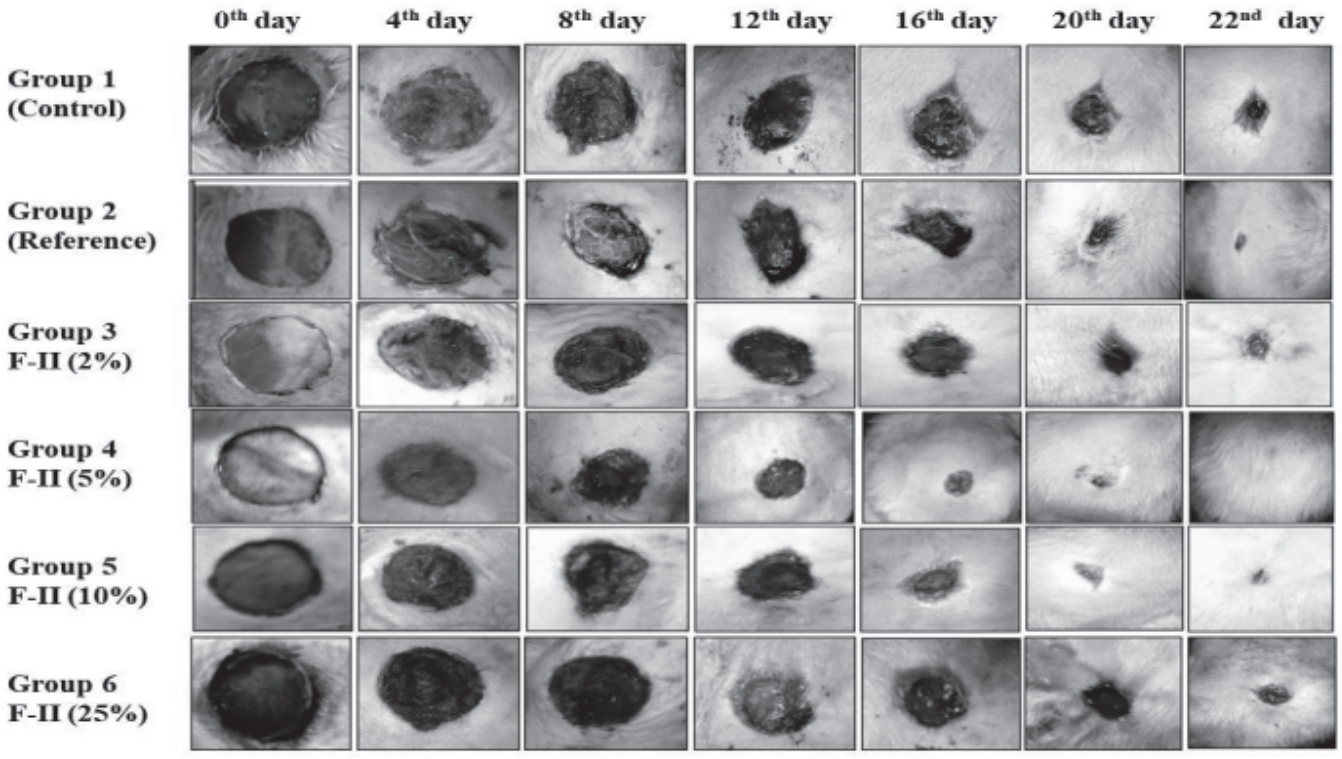

Fig. 3: Images of wound areas in different groups of experiment III at different time intervals

in control (Table 5 and Fig. 3). The 5% F-III showed significantly faster completely wound contraction at 18th day with absence of scar compared to F-I (20th day) and F-II (22nd day) and reference group.

Discussion

Good wound care is essential for effective management. Wound healing involves replacing damaged skin tissue through biological processes like clot formation and tissue generation. Antiseptics may have a toxic effect on tissues and are not suitable for open wounds. It's advised to use them with caution as their toxicity might outweigh any benefits[13]. Some reports suggest that they may exhibit cytotoxicity and are advised against their application on open wounds. Wound healing agents primarily function in the Inflammatory and Proliferative phases[17]. Common antiseptics may have a toxic effect on tissues and caution is advised when using them. It's recommended to combine traditional and modern wound healing agents for optimal care.

Various studies have reported an improved wound-healing process following topical application of herbal products[31]. The results of the present study demonstrated wound healing abilities of ethanolic extracts of a specified set of plants/herbs as Formulations in excision wounds, as evident by the generation of granulated tissue and remarkable increase in the rate of wound contraction compared to the reference formulation *viz.,* Betadine. Phytochemicals-based formulations appear to influence one or more stages resulting in faster wound closure when compared to the control and reference groups.

Medicinal plants have different phytochemicals as secondary metabolites. Phytochemicals such as flavonoids, phenols and tannins present in phytochemical formulations are shown to possess anti-microbial, anti-inflammatory, anti-oxidant activities and are thus responsible for wound healing activity[5], because Tannins have strong astringent property and promote capillary vasoconstriction, which decrease vascular permeability and cause a local anti-inflammatory effect[23]. Flavonoids are well known for their antioxidant potential and antibacterial properties which promote the wound healing process by wound contraction, increased rate of epithelialization and raise the level of hydroxyproline supporting homeostasis[19].

The results of this study seem to confirm the use of F-III consisting of *T. erecta, T. procumbens, A. indica, F. benghalensis, C. longa* and *A. vera* for faster and effective treatment of excision wounds followed by F-I and F-II. The extracts of these plants can be developed into phytomedicines for wider application in the management of wounds.

Conclusion

After conducting experiments on excision wounds, it can be concluded that the topical application of all phytochemical formulations -I, II, and III separately shows significant wound healing activity. This was evidenced by faster generation of fibrocollagenous tissue, neovascularization, epithelialization, and anti-bacterial functions. F-III (5%) proved to be superior in achieving faster wound healing than the other formulations (F-I and F-II) studied. Since *Tagetes erecta, Tridax procumbens, Azadirachta indica, Ficus benghalensis, Aloe vera* and *Curcuma longa* are widely available and abundant, they could provide a fairly economical wound healing agent for wider application in wound care management. Further studies are required to identify the active compounds participating in processes of wound healing.

References

1. Abhishek J, Denna P, Divya B. A phytochemical screening of the ethanolic extract of the *Aloe Vera* Gel. *Int. J. Science and Research (IJSR).* 2019; **8**(10): 2319-7064.

2. Ajmary S, Mahadi H, Marzia RMD, Mahmudul A. Healing potentials of Marigold flower (*Tagetes erecta*) on full thickness dermal wound in caprine model. *J. European Research*. 2021; **7**(4): 332-339.

3. Alhakmani F, Kumar S, Khan SA. Estimation of total phenolic content *in-vitro* antioxidant and anti-inflammatory activity of flowers of *Moringa oleigera*. *Asin. Pac. J. Trop. Biomed.* 2013; **3**(8): 623-627.

4. Diegelmann RF, Evans MC. Wound healing: An overview of acute, fibrotic and delayed healing. *Front Biosci.* 2004; **9**: 283–289.

5. Ghosh PK, Gaba A. Phyto-extracts in wound healing. *Pharma. Sci.* 2013; **16**: 760-820.

6. HEES CT. Understanding the barriers to healing. *Adv. Skin Wound Care.* 2012; **25**(5): 240.

7. Lakshana S, Vijaylakshmi S, Dinakar J, Asok KK. Effect of *Tagetes erecta* gel on experimentally induced oral ulcer model in rats. *Int. J. of Res. Pharma. Sci*. 2020; **11**(2): 1844-1848.

8. Mehmood A, Javid S, Khan MF, Khawaja Shafique Ahmad KS, Mustafa A. *In vitro* total phenolics, total flavonoids, antioxidant and antibacterial activities of selected medicinal plants using different solvent systems. *BMC Chemistry*. 2022; **16**(64).

9. Meurer MC, Mees M, Mariano LNB, Boeing T, Somensi LB, Mariott M, Da Silva RCMVAF, Dos Santos AC, Longo B, Santosh Franca TC, Klein-Junjor LC, De Souza P, De Andrade SF, De Silva LM. Hydroalcoholic extract of *Tegetes eracta* L. flowers, rich in the carotenoid lutein, attenuates inflammatory cytokine secretion and improves the oxidative stress in a stress in an animal model of ulcerative colitis. *Nutrition research.* 2019; **66**: 95-106.

10. Mohmad I, Jyoti NS, Mehnaz K, Mohammad A. Standardization And Wound-Healing Activity of Petroleum, Ethanolic and Aqueous Extracts of *Ficus benghalensis* leaves. The International Journal of Pharmaceutical Chemistry*. Russian Original.* 2021; **54**(10) : 1052-1062.

11. Molazem Z, Mohseni F, Younesi M, Keshavarzi S. *Aloe vera* gel and cesarean wound healing; a randomized controlled clinical trial. *J. Glob J Health Sci.* 2014; **7**(1): 203-09.

12. Muhammad A, Usman RB, Bala HA, Isyaku IM. Quantitative and qualitative phytochemicals and proximate analysis of *Aloe vera* (*Aloe barbadensis* Miller). *The Int. J. Advanced Academic Research. Sciences, Technology and Engineering*. 2020; **6**: 2488-9849.

13. Murthy S, Gautam MK, Shalini G, Purohit V, Sharma H, Goel RK. Evaluation of *in vitro* wound healing activity of *Bacopa monniera* on different wound healing model in rats. *Int. J. Biomed Research.* 2013;1-9.

14. Naveen KC, IKE R, IRRA R. Effect of Neem Leaves Extract (*Azadirachta Indica*) on Wound Healing. *Althea Medical*. 2015; **2**(2) : 199-203.

15. Ogunlowo OP, Arimah BD. Phytochemical analysis and comparison of *in-vitro* antimicrobial activities of the leaf, stem bark and root bark of *Ficus benghalensis. IOSR Journal of Pharmacy.* 2013; **3**(4): 33-38.

16. Oguwike FN, Onubueze DPM, Ughachukwu P. Evaluation of activities of Marigols extract of wound healing of Albino Wister Rat. *J. Dental and Medical Sciences.* 2013; **8**(5): 67-70.

17. Ortega-Llamas L, Quiñones-Vico MI, García-Valdivia M, Fernández-González A, Ubago-Rodríguez A, Sanabria-De La Torre R, Arias-Santiago S. Evaluation in skin cell lines after treatment with common Antiseptics for Clinical use. *Cells.* 2022; **20**(11): 1395-1402.

18. Pazyar NR, Yaghoobi R, Rafiee E, Mehrabian A, Feily A. Akin wound healing and phytomedicine: A review. *Skin Pharmcol, Physiololy.* 2014; **27**: 303-310.

19. Pratibha S, Samriti F. Potential of herbal plants in wound healing. *Pharmaceutical negative results*. 2022; **13**(8): 460-471.

21. Rajesh L, Thangapazham S, Radha KM. Phytochemicals in wound healing. *Aadvance in wound care*. 2013; **5**(5) : 230-241.

22. Ramya KB, Hema LD, Chetash CH, Lakshmi M. Preparation and evaluation of turmeric herbal formulation. *Int. J. Green and Herbal Chemistry.* 2015; **4**: 286-295.

23. Sapna S, Anju D, Sanju N. Traditional Indian medicinal plants with potential wound healing activity: A review. *Int. J. Pharmaceutical Sciences and Research.* 2016; **7**(5): 1809-1819.

24. Sembian S, Kalidass S, Jeevan RDS, Femina W, Pemila ECR. Evaluation of wound healing activity of *Acacia caesia*in rats. *Wound Med.* 2015; **7** : 1-7.

25. Sharma A, Khanna S, Kaur G, Singh I. Medicinal plants and their components for wound healing applications. *Futur J. Pharm. Sci.* 2021; **7**(53) : 1-13.

26. Shedoeva A, Leavesley D, Upton Z, Fan C. Wound healing and the use of medicinal plants. *Evidence-based complementary and alternative medicine.* 2019; **22** : 1-30.

27. Siddhu N, Saxena J. Quantification of total phenolic and total flavonoid content of extracts of *Tagetes erecta* flowers. *Asian J. Pharmaceutical and Clinical Research.* 2017; **10**(6): 328-330.

28. Subramanian A, Samuel T, Selvaraj D, Shameem R, Grace M. GC-MS analysis of bioactive compounds of *Curcuma longa* Linnaeus (Zingiberaceae) rhizome extract. *Pharmacognosy and Phytochemistry*. 2019; **8**(6): 49-52.

29. Sujata K. Qualitative and quantitative phytochemical screening of *Azadiracta indica* Juss. Plant parts. *Int. J. Appl. Sci. Biotechnol.* 2021; **9**(2): 122-127.

30. Syed A, Benit N, Alyousef AA, Alqasim A, Arshad M. *In-vitro* antibacterial, antioxidant potentials and cytotoxic activity of the leaves of *Tridax procumbens*. *Saudi J. Biological Sciences.* 2020; **27**: 757–776.

31. Thakur R, Jain N, Pathak R, Sandhu SS. Practices in wound healing studies of plants. Evidence-based complementary and alternative medicine: *eCAM,* 2011, 438056.

32. Yogendra S, amit G Pusphendra K. *Tagetes erecta* (Marigold)-A review on its phytochemical and medicinal properties. *Curr. Med. Drug. Res*. 2020; **4**(1) : 1-6.

33. Yogesh PT, Biswaeep D, Tania P, Deepali Y, Tania P, Kishori GA. Evolution of wound healing potential of aqueous and ethanolic extracts of *Tridax procumbens* linn. In *wistar* rats. Asian pharm. *Clin. Res.* 2012; **5**(4): 140-145.