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Validation of Traditional Therapeutic Claims through Phytochemical Screening and Antibacterial Assessment: A Study on Mahakaal (*Trichosanthes tricuspidata* L.) From Similipal Biosphere Reserve Forest, Odisha, India

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Abstract: Similipal Biosphere Reserve forest is situated in the district Mayurbhanj, Odisha, enriched with the different types of vegetations along with aboriginals. These aboriginals have unique skills in using traditional therapeutic medicines. They use wild plant and their parts in traditional herbal formulations to cure different diseases. *Trichosanthes tricuspidata,* locally known as Mahakaal is very common to be used as herbal medicine. Fruits of Mahakaal have sound traditional therapeutic values, they have been used against asthma, skin infections, muscular pain and killing the head lice. Phytochemical screening of fruit extracts revealed the presence of major bioactive compounds such as Tannin, Saponin, Flavonoids, Phenolic compounds, Terpenoids etc which indicate its sound pharmacological properties. Antibacterial assessment of fruit extracts also showed excellent activity against two Gram-positive and three Gram-negative bacteria. Methanol extracts showed highest zone of inhibition (1.51 cm) against *Streptococcus pyogenes* caused skin infections. The experimental works validate the traditional therapeutic claims.

Keywords: Similipal Biosphere Reserve, Mahakaal, Traditional therapeutic values, bioactive compounds, antibacterial activity, validation

I. INTRODUCTION

A Similipal Biosphere Reserve (SBR) forest is the only Biosphere Reserve of the state of Odisha. The name “Similipal” probably derived from abundance of Simili tree (*Salmalia malbarica* DC. Schott & Endl) in this biosphere [1]. SBR covers an area of 5569 Km^2, situated (20° 17´ - 22° 34´ N and 85° 30´ – 87° 10´ E) in the centre of Maurbhanj district in

the state of Odisha [2, 3, 4]. Biosphere is enriched with moist deciduous forest (Chahala, Jenabil, Nawana, Bakua etc), dry deciduous forest (Gurguria, Barheipani, Kalyani, Sanuski, Asatakumar, Kusumi, Rajpal, Hatibadi etc) and grass land (Chahala, Ligirdha etc). SBR is rich in wild medicinal plants along with the harbor of orchids [3, 5, 6]. It is also populated with different tribal communities such as Hill-Khadia, Mankidia, Ho, Munda, Bathudi etc. having their own socio-cultural habits and skills [7]. They are known to be primitive medical practitioners in the area as they use various wild phytoresources to cure diseases [8]. Hill-Khadia is a primitive tribe, having unique skills of collection of wild medicines [9, 10]. The plants used as traditional medicine & ailments by these tribes either are found in the peripheral, buffer zones, core or in the locality of tribal villages or hamlets [11]. The plants found in and around the villages are commonly used by them as traditional medicines. The plants mostly belong to the families like Cucurbitaceae, Rutaceae, Convulvulaceae, Moraceae, Myrtaceae, Malvaceae, Solanaceae, Fabaceae etc. Among these families, plants of Cucurbitaceae are quite common in villages of buffer and peripheral zones of SBR. *Luffa actungula, Luffa agyptica, Cucumis melo, Cucumis mexima, Cocinia grandis, Tricosanthes cucumerina, Tricosanthes tricuspidata and Momordica charantia* belonging to the family Cucurbitaceae, are mostly wild in nature and commonly used to cure different diseases by these people. *Tricosanthes tricuspidata*, (Plate1) locally called as “Mahakaal” is popular of this area due to its attractive red coloured fruits [12]. *T. tricuspidata* is a common medicinal plant used by the aboriginals of SBR. Fruits are used against asthma, skin infections, inflammation and joint pains. However, very less documentation / reports are available in literature about the medicinal and pharmacological values of *T. tricuspidata* fruits. Therefore, an attempt was made to document the ethnobotanical values collected from tribal communities of SBR and to validate their claims through phytochemical screening and antibacterial assessment.

II. MATERIAL AND METHODS

The present study is based on the field survey for collecting ethnobotanical data and lab work for investigation of bioactive compounds and antibacterial activity. The details of materials used, experimental methods and techniques adopted during the course of the entire investigation are described below.

II.1. Assessment of Ethnobotanical data

The results presented here based on the fieldwork conducted with the rural and tribal communities of adjoining areas of SBR (Jashipur, Karanjia, Bisoi, Kendumundi and Padampur) during 2010-2013. The methodological framework was followed as per standard technique of ethno-biological approaches [13]. The information on plant used as traditional medicine against different diseases and disorders were collected through questioners with different rural and tribal communities [14, 15]. The pharmacological and medicinal properties of *T. tricuspidata* were confirmed by cross check with informants. Plant species was confirmed with the Flora’s Book and published article [16, 12].

Plate 1: Collection of Plant materials, 1: Fruits of T. tricuspidata, 2: Collection of fruits at Similipal Biosphere Reserve, 3: Collection of ripen fruits at Hatibadi village

II.2. Collection of plant materials

Fruits of T. tricuspidata were collected from the village Padampur, Hatibadi and Kalikaparsad peripheral area of SBR. They were washed properly, cut into small pieces and left for air-drying. The dried materials were crushed to powder with mechanical device and kept in airtight container for phytochemical screening and antibacterial activity (Plate1).

II.3. Preparation of plant extracts

As per polarity index four solvents (n-butanol, acetone, methanol and aqueous) were selected for extraction. Extraction was done using Soxhlet apparatus followed by standard method [17].

II.4. Qualitative detection of bioactive compounds

Phyto-chemical experiments were carried out on n-butanol, acetone, methanol, and aqueous extracts of *T. tricuspidata* fruits using standard procedure to identify the bioactive compounds as described by different workers [18, 19, 20, 21].

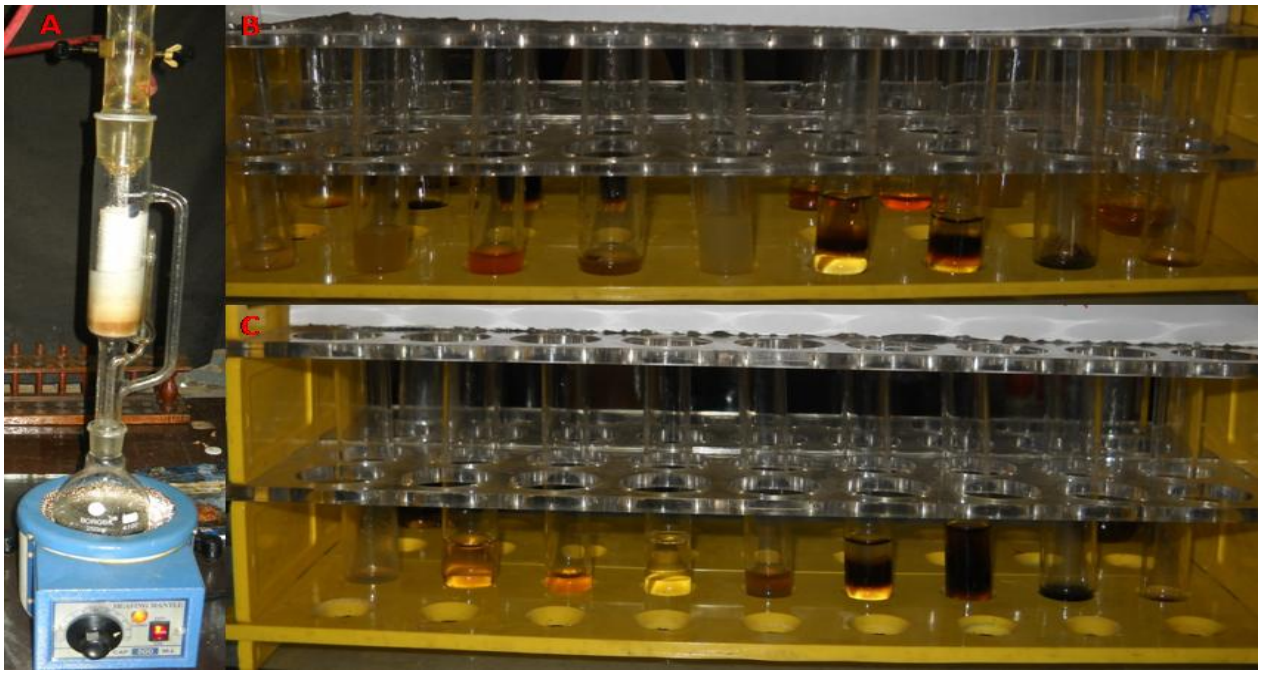

Plate 2: Extract preparation and qualitative estimation of bioactive compounds present in fruit extracts

II.4.1. Test of Tannin

0.5 gm of dried powder sample was boiled in 10 ml of distilled water and filtered with Whatman 42 filter paper. 2 ml of filtrate was taken in a test tube and 3-5 drops of 0.1 % ferric chloride solution were added. The brownish green or blue black colouration indicated the presence of tannins.

II.4.2. Test for Alkaloids

0.5 gm of crude extract was mixed with 5 ml of 1% aqueous HCl on water bath and then filtered. 2-5 drops of Dragendorff's reagent were added in the filtrate. The occurrence of orange-red precipitate was indicated the presence of alkaloids in the sample extract.

II.4.3. Test for Saponin

0.5 gm of the dried powder was boiled in 15 ml of distilled water and filtered with Whatman 42 filter paper. 5 ml of filtrate was mixed with 2 ml of normal distilled water and shaken vigorously. The stable persistent froth indicated the presence of saponins.

II.4.4. Test of Flavonoids

6 ml of dilute ammonium solution was added to portion of the aqueous filtrate of plant extract followed by addition of concentrated sulphuric acid. A yellow colouration indicated the presence of flavonoids.

II.4.5. Test of Terpenoids

6 ml of extract was mixed in 2.5 ml of chloroform and then 3 ml of concentrated sulphuric acid was added. A reddish-brown colouration of interface indicated the presence of terpenoids.

II.4.6. Test of Cardiac Glycosides

5 ml of crude extract was treated with 3 ml of glycial acetic acid. Then 3-5 drops of 1 % ferric chloride solution was added followed by 2 ml of concentrated sulphuric acid. Formation of brown interface indicated the presence of cardiac glycosides.

II.4.7. Test of Phenolic compounds

0.5 gm of extract was treated with 3-5 drops of 1 % ferric chloride solution. Formation of bluish black colouration indicated the presence of phenolic compounds.

II.4.8. Test of Phytosterols

0.5 gm of extract was treated with chloroform and then filtered. The filtrate is treated with 3-5 drops of concentrated sulphuric acid and shakened. The mixture was left for 5 minutes. The appearance of Golden yellow colour indicated the presence of Phyto-sterols.

II.4.9. Test for Steroids

2 ml of plant extract was dissolved in 5 ml chloroform and then 5 ml of concentrated sulphuric acid was added. Formation of 2 phases (upper red and lower yellow with green fluorescence indicated the presence of steroids.

II.5. Antibacterial activity

The n-butanol, acetone, methanol and aqueous extracts of *T. tricuspidata* fruits were screened for antibacterial activity against two Gram-positive bacteria *Streptococcus mutans* (MTCC *497) and *Streptococcus pyogenes* (MTCC 1926); three Gram-negative bacteria *Vibrio cholera* (MTCC 3906), *Shigella flexneri* (MTCC 1457) and *Salmonella enterica typhi* (MTCC 1252). All these used MTCC (Microbial Type Culture Collection) bacterial strain were collected from Institute of Microbial Technology (IMTECH), Chandigarh. Antimicrobial activity was done using Agar Well Diffusion assay adopted
standard method [22] with slight modification. Wells (6 mm) were made using sterile borer. Stock solutions of samples were prepared in 100 % DMSO (dimethyl sulfoxide) (Sigma) and twofold serial dilutions were made in amount of 100 µl per well ranged from 0.25 and 0.5 mg / ml. 100 µl of samples were added by sterile syringes into the wells in three above mentioned concentration and allowed to diffuse at room temperature for 2 h. Plates were incubated at 35 ± 2°C for 18-24 h. Ampicillin served as standard drug control. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings (diameter of zone of inhibition in cm) were taken and the mean ± SD values (diameter of zone of inhibition) were recorded**.**

II.6. Data analysis

Mean and SD (standard deviation) was performed to evaluate triplicate values of zone of inhibition (cm) of samples using Excel, Microsoft Corporation-2010, US.

III. RESULTS AND DISCUSSION

T. tricuspidata is very common plant in the peripheral and buffer regions of the SBR. It has a popular name in the locality “Mahakaal” dedicated to lord “Shiva” (Plate1:1). The ethnobotanical survey indicated its sound therapeutic values among the aboriginals of SBR. The aqueous paste of fruits is very effective in headache. The Bathudi tribe of Hatibadi uses it frequently as natural head balm (Table 1). Among the tribe of Kadlibadi, fruits paste are

used to cure mouth inflammation of domestic cattle. Fruits paste is very good for reducing macular swelling and pain. The tribe of Padampur used it against different types of skin infections and also against itching (Table 1). Fruits are very popular among the tribal women as it is used to kill the head lice and to cure dandruff problems. The medicinal values of this plant reported by different researchers throughout the world [16, 23, 24, 25]. The qualitative screening of bioactive compounds revealed that fruits of *T. tricuspidata* are rich in secondary metabolites, which is indicative of its pharmacological values. Terpenoids present in n-butanol extract of *T. tricuspidata* fruit showed the analgesic properties [26]. Flavonoids present in acetone, methanol and aqueous extracts (Table 2) showed its antibacterial [27], anti-cancer [28], and anti-inflammatory activity [29]. Presence of tannin and saponin in aqueous extract (Table 2) showed the anti-microbial [30, 31] activity and toxic properties [32] of *T. tricuspidata* L. fruits.

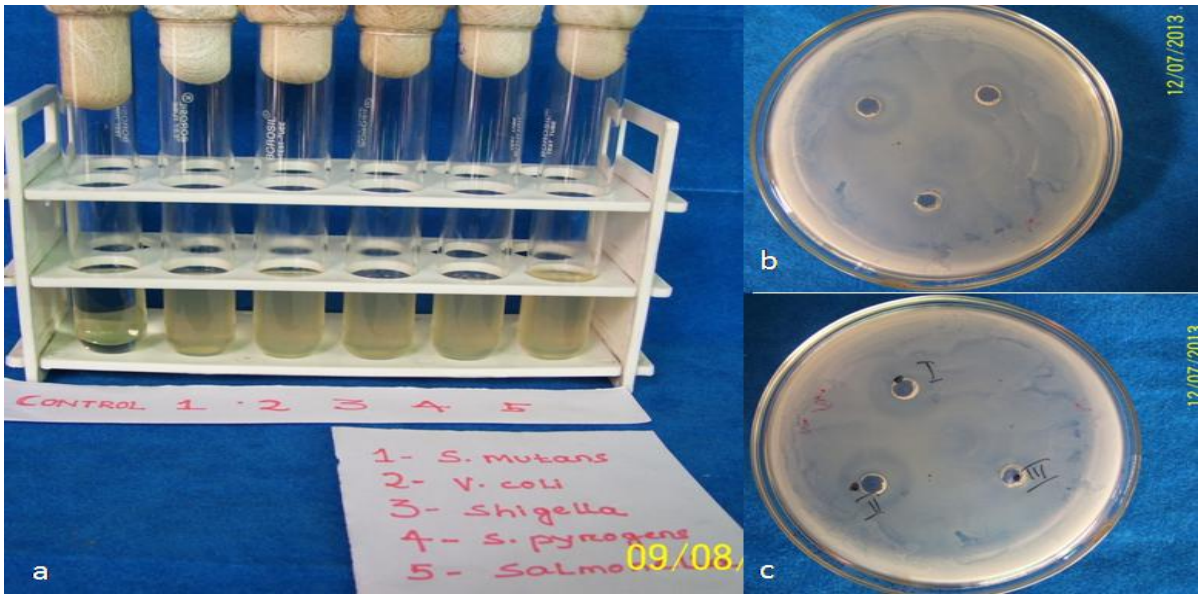

Plate 3: Antibacterial activity, a: broth with control; b & c: zone of inhibition of methanolic extract against MTCC 1926.

The anti-bacterial activity showed excellent zone of inhibition against two gram positive and three gram negative bacteria. At concentration 0.25 mg/ml, the methanol extract of *T. tricuspidata* L. fruit showed highest zone of inhibition against MTCC 1252 (1.18 cm), MTCC 1457 (1.25 cm), MTCC 3906 (1.28 cm), MTCC 1926 (1.28 cm) and MTCC 497 (1.41 cm) followed by acetone extract, n-butanol and aqueous extract (Plate 3).

At the concentration 0.5 mg/ml the methanolic extract showed highest activity than other extracts (Table 3). The ethnobotanical claims that fruits preparations are used against various diseases are validated by the present experiment. The presence of bioactive compounds correlated to the therapeutic properties of fruits. Presence of flavonoid might be responsible to swellings and muscular pain [39]. Presence of tannin and saponin might be responsible for skin infections [40] and antimicrobial activity [30] (Table 4).

***Table 1**: Ethnobotanical values of Mahakaal (T. tricuspidata L.) fruit among the rural and tribal communities of Similipal Biosphere reserve and its adjoining areas*

Informants	Sex	Age	Collection site	Forms of use(s)	Mode of use(s)
Abhiram Bisad	Male	45	Hatibadi	Paste, external	Paste of two matured green fruits is macerated with about 10 ml of hot water. Macerated paste is then applied thickly on forehead to reduce headache.
Laxaman soi	Male	52	Hatibadi	Paste	Dry Fruits are macerated with fruit oil of *Azadir achta indica* (Neem) and camphor. Macerated paste is applied on head and kept it about 30 minutes to kill lice thrice in a week before bath.
Bajraj Chandra Nayak	Male	38	Hatibadi	Paste	Green newly 10 days fruits are cut in small pieces. Pieces are kept in small amount of water and kept overnight. Swelled fruits are macerated and make paste with same water. Paste is applied as natural ointment on swelling parts of the body to reduce pain and inflammation.
Morgad Nayak	Male	51	Kadlibadi	Paste	Seeds of ripen fruits are washed and dried. Dried seeds are powdered using traditional mortar pestle of stone. Approx 200 grams of powder are macerated with water and make paste. Paste is applied on outer parts of mouth of cattle to cure swelling.
Jairam Futi	Male	44	Kadlibadi	Paste	Ripen fruits are dried with pulp and seeds. Dried fruits are powdered using the traditional grinder. Approx 100 gm powder is macerated with about 10 ml seed oil of *Ricinus cumunis* (Joda) to cure itching on skin.
Rama Ho	Male	40	Padampur	Paste	Black pulp of ripen fruits are dried and powdered. Powder are mixed with seed oil of *Pongamia pinnata* (Karanja) and made paste. Paste is applied on lesions of fungal infections on feet, particularly during rainy seasons.
Bnamali Ho	Male	41	Padampur	Paste	Dry Fruits are macerated with fruit oil of *Azadirachta indica* and camphor. Macerated paste is applied on head and kept it about 1h to kill lice thrice in a week before bath.
Balram Munda	Male	55	Padampur	Paste	Dry Fruits are macerated with fruit oil of *Azadirachta indica* and camphor. Macerated paste is applied on head and kept it about 30 minutes to kill lice thrice in a week before bath.

***Table 2** : Qualitative analysis of bioactive compounds present in fruits of Mahakaal (T. tricuspidata L.)*

Solvents	bioactive compounds
n-butanol	Terpenoids
Acetone	Flavonoids, Phenolic compounds
Methanol	Flavonoids. Alkaloids, Steroids, Phenoilc compounds
Aqueous	Tannin, Saponin, Flavonids, Phenolic compounds

The presence of saponin might be responsible to kill the lice. The antibacterial activity of methanolic extract against *Streptococcus pyogenes* (MTCC 1926) showed that fruits are effective against the various skin infections (Figure 1) as *S. pyogenes* are responsible for skin infections [41].The experimental results validated the traditional therapeutic claims (Table 4) to be used fruit preparations against aforesaid diseases.

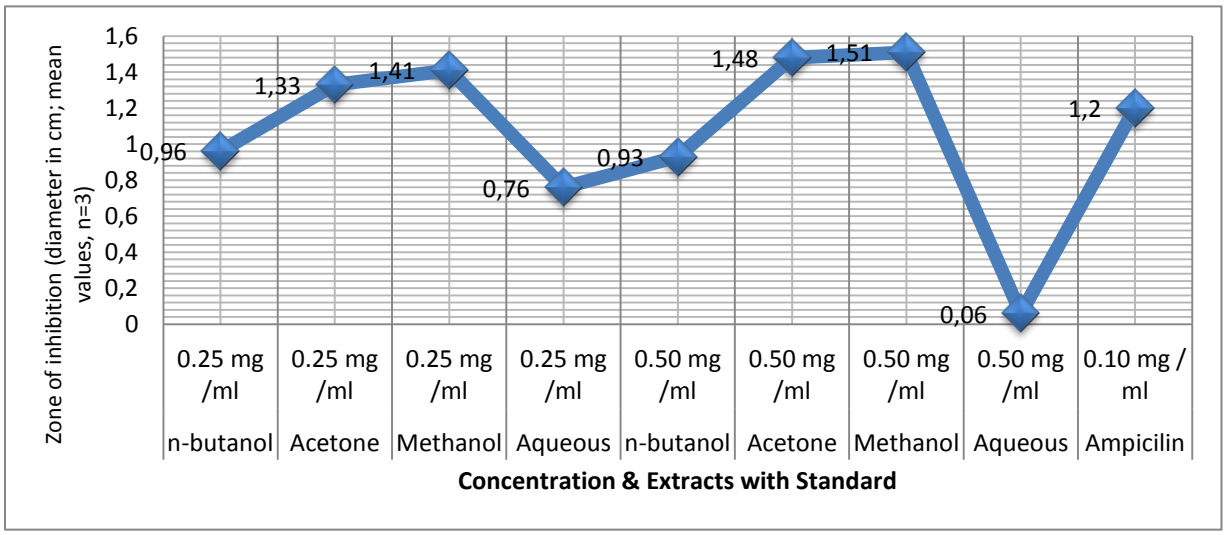

***Figure 1**: Antibactrial activity against MTCC 1926*

***Table 3**: Antibacterial activity of fruit extracts of Mahakaal (T. tricuspidata L.)*

Antibacterial activity (Agar well diffusion assay, Zone of inhibition, cm; mean ± SD; n=3)					
Strain	**n-butanol**	**Acetone**	**Methanol**	**Aqueous**	**Concentration**
MTCC 1252	0.73 ± 0.02	1.20 ± 0.02	1.18 ± 0.07	0.76 ± 0.02	0.25 mg/ml
MTCC 1457	0.91 ± 0.05	1.21 ± 0.02	1.25 ± 0.05	0.76 ± 0.02	
MTCC 3906	0.88 ± 0.02	1.16 ± 0.02	1.28 ± 0.02	0.78 ± 0.02	
MTCC 1926	0.96 ± 0.02	1.33 ± 0.02	1.41 ± 0.02	0.76 ± 0.02	
MTCC *497	0.95 ± 0.05	1.16 ± 0.02	1.23 ± 0.02	0.86 ± 0.02	
MTCC 1252	0.96 ± 0.02	1.40 ± 0.05	1.51 ± 0.02	0.98 ± 0.02	0.5 mg/ ml
MTCC 1457	0.98 ± 0.02	1.41 ± 0.02	1.43 ± 0.02	0.95 ± 0.05	
MTCC 3906	0.96 ± 0.02	1.46 ± 0.05	1.48 ± 0.02	0.98 ± 0.02	
MTCC 1926	0.93± 0.05	1.48 ± 0.02	1.51 ± 0.02	1.06 ± 0.05	
MTCC *497	0.95 ± 0.05	1.18 ± 0.07	1.16 ± 0.11	0.96 ± 0.02	
Ampicilin (zone of inhibition, cm, mean ± SD; n=3)					
MTCC 1252	1.10 ± 0.00				0.1 mg / ml
MTCC 1457	1.40 ± 0.00				
MTCC 3906	1.20 ± 0.00				
MTCC 1926	1.20 ± 0.00				
MTCC *497	1.40 ± 0.00				

(MTCC: Microbial Type Culture Collection; SD: standard deviation; mg: milligram; ml: milliliter; MTCC 3906: *Vibrio cholera*; MTCC 1252: *Salmonela enteric typhi*; MTCC 1457: *Shigella flexneri*; MTCC 1926: *Streptococcus pyogenes*; MTCC 497: *Streptococcus mutans)*

***Table 4**: Validation of traditional claims against Mahakaal (T. tricuspidata L.) fruits*

Traditional claims	Correlation with bioactive compounds	Correlation with antibacterial activity	Supporting literature
Skin infections	Presence of saponin in aqueous; tannin in aqueous; flavonoids in acetone and methanol extracts might be responsible.	Methanolic extract showed highest zone of inhibition (1.51 ± 0.02) against *Streptococcus pyogenes* (MTCC 1926).	Mohan and Kalidas, (2010) [33]; Okwe & Okwe, (2004) [34]; Kumar *et al.,* (2013) [35].
Kill lice	Presence of saponin in aqueous extract might be responsible to kill lice due to foam forming capacity (needs further study)		
Reduce swelling	Presence of flavonoids in acetone and aqueous extracts might be responsible to reduce swelling.		Musa *et al.,* (2009) [36]
Reduce pain	Presence of flavonoids in acetone, methanol and aqueous extract; tannin in aqueous extract might be responsible		Majumadar *et al.,* (2008) [37]; Gloria and Soloman, (2008) [38]; Musa *et al.,* (2009) [36].

IV. CONCLUSION

The present study highlights the medicinal values of Mahakaal (*T. tricuspidata* L.) fruits and its uses among the populace of a protected area, Similipal Biosphere Reserve. The studies scientifically validate the tribal claims on the ethnomedicinal uses of fruits. Further present study suggest to isolate the bioactive compounds which actually active against above mentioned claims to formulate drugs. Such study also highlights the medicinal values of the wild plants and gives attention towards the possible awareness and to make policies for conservation of such wild resources.

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