Indian Journal of Natural Products and Resources
Vol. 13(1), March 2022, pp. 60-66

Green extraction and *in vitro* anti-mycobacterial activity of *Hydrocotyle sibthorpioides* Lam. and *Carica papaya* L. leaves collected from Assam, India

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Received 01 August 2020; Revised 21 February 2022

The traditional healers of different parts of India use goat urine for the treatment of tuberculosis. On the basis of ethnomedicinal claims, two plant species namely *Hydrocotyle sibthorpioides* and *Carica papaya* were extracted with raw (fresh) and photo-activated goat urine as a menstruum. The present study reports the *in vitro* antimycobacterial activity of the leaf extracts of *H. sibthorpioides* and *C. papaya* against *Mycobacterium smegmatis* (ATCC 700084 /Mc2155 strain). It was observed that the photo-activated goat urine and raw goat urine leaf extracts could inhibit *M. smegmatis*. Among all the four extracts, the extract of *C. papaya* using photo-activated goat urine showed the highest antimycobacterial activity against *M. smegmatis.*

Keywords: Ajamutra, Anti-tubercular, Ethnomedicine, Goat urine, Maceration, Northeast India.

IPC code; Int. cl. (2021.01)- A61K 36/00, A61K 36/25, A61K 127/00, A61P 31/00

Introduction

The Indian Traditional System of Medicine, primarily the *Ayurvedic* system elucidates the use of goat urine in boosting the general health of an individual[1] and is also reported to be used in the treatment of tuberculosis. Several tribes such as the Saharia tribe of Rajasthan, the tribal inhabitants of Attapady hills and other tribes hailing from different parts of India orally administer goat urine as a medication for tuberculosis (TB)[2]. TB which is caused by *Mycobacterium tuberculosis* is considered a highly infectious disease with an annual occurrence rate of more than 9 million and it is reported to have caused about 1.3 million deaths in 2014[3]. A large number of the world's population is estimated to be infected by it and is regarded to be a major cause of human fatality in the developing world[4].

With the emergence of efficacious antimycobacterial agents between 1950 and 1970 like ethambutol, isoniazid, rifampicin, and streptomycin there was a substantial reduction in the number of TB cases all over the world, particularly in developing nations[5]. Although these drugs have been developed to treat tuberculosis, they are not always efficient due to the emergence of drug-resistant strains[6]. It has been reported that there is an increase in cases of Multi-Drug Resistant TB (MDR-TB) and Extensively Drug-Resistant TB (XDR-TB) have deteriorated the situation and constituted a severe health risk. Therefore, potent, novel and cost-effective anti-tubercular drugs with low toxicity are essentially required to encounter the risk of TB[7].

India is a diverse land with exclusive knowledge about medicinal plants and their uses in treating several diseases[8]. These plants play a crucial role in formulating herbal drugs and provide notable health care aid to a vast section of individuals, especially in developing countries[9]. These medicinal plants have been widely used for healing disease for many centuries[10]. Out of the 20,000 medicinal plants that have been recorded to date, the traditional healers use 7000 to 7500 plants to cure various diseases[11]. A wide range of medicinal plants has been traditionally used to eradicate the symptoms of tuberculosis. Of the 17,500 higher plant species found in India, about 365 species have been estimated to be used for antimycobacterial activities[5]. Researchers have isolated the natural phytochemicals that are essentially required to encounter the risk of MDR-TB. The isoquinoline alkaloid berberine isolated from the roots of *Berberis aristata* and its derivatives [13-

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benzyl (3–6), 13-allyl (7, 8), 8-(2-oxopropyl) (2), and 9-hydroxy (9)] have attributed the anti-tubercular activity against *M. tuberculosis*. Berberine (MIC, 16 µg/mL) anti-tubercular activity is probably due to the 13-benzyl and allyl substitution in the molecule[12–15].

Hydrocotyle sibthorpioides Lam. (Araliaceae) and *Carica papaya* L. (Caricaceae) are well-known ethnomedicinal plants. These plants are extensively used in the Indian traditional system to cure ailments. The leaves of *H. sibthorpioides* are traditionally used to treat dysmenorrhoea[16]. Freshly crushed leaves are also locally applied to the affected part in the treatment of carbunculosis[17]. It was also reported to heal rheumatic troubles and skin diseases, including syphilis and liver complaints[18]. Studies have also described that the species of *Hydrocotyle* could prohibit the growth of transplanted tumours in mice, such as hepatic carcinoma (Hep), sarcoma (S180) and uterine cervical carcinoma (U14)[19]. It was also reported to have antimicrobial and antioxidant activities[20]. The leaf extracts of *C. papaya* have been studied for a vast range of biological and pharmacological activities like antioxidant, antibacterial, antifungal, antiamoebic, wound-healing, antihelmintic, antiulcerogenic, hypolipidemic, antihypertensive, diuretic and antifertility activities[21]. The latex of the plant is utilized as an analgesic in tooth decay. The ripe fruit and seeds of *C. papaya* are used in diarrhoea and as anthelmintics. A decoction of the unripe fruits is taken orally in jaundice[22,23]. According to *Astanga Samgraha* (an ancient authoritative text on *Ayurveda*) of Acharya Vagbhata[24,25], goat urine can be used to treat the ailment of cough, respiratory difficulties, dyspnea, earache, jaundice, oedema and anaemia[26]. The different tribal communities in the Attappady hills of Western Ghats utilize goat urine for the treatment of tuberculosis[27]. Goat milk is externally used for the treatment of eye diseases. The pulmonary tuberculosis can be cured by staying in close vicinity in the company of goats and by drinking goat's milk[28]. The proteins present in goat urine have potential *in vitro* antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*[1]. Moreover, it is externally applied to treat skin infections such as itching, ringworms, dermatophytosis or herpes and tinea infections[26].

In view of the potential activities of goat urine, *H. sibthorpioides*, and *C. papaya*, the present study was designed and carried out to evaluate the antimycobacterial activity of raw goat urine extract and photo-activated goat urine extract of *H. sibthorpioides* and *C. papaya*.

Materials and Methods

Raw materials

Rifampicin was procured commercially (Himedia, Mumbai, India), and Berberine (GLR Innovations, New Delhi) was supplied by M/S Vinayak Drugs & Surgicals, Dibrugarh, Assam. *Mycobacterium smegmatis* (*M. smegmatis*) (ATCC 700084 / Mc^2155) strain was procured from the Center for Biotechnology & Bioinformatics, Dibrugarh University, Dibrugarh (India).

Plant collection and identification

The leaves of the plant *H. sibthorpioides* and *C. papaya* were collected from Hatiali, Dibrugarh, Assam, India, in December 2017 and February 2018.The plants were identified and authenticated at the Botanical Survey of India (BSI), Eastern Regional Centre, Shillong, Meghalaya, India as *H. sibthorpioides* (BSI/ERC/Tech./Plant Iden./2018/115 dt. 22.05.2018) and *C. papaya* (BSI/ERC/Tech./Plant Iden./2018/114 dt. 22.05.2018). The herbarium specimen was deposited in the Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh.

Drying of the plant materials

The leaves of *H. sibthorpioides* and *C. papaya* were shade dried at room temperature until it was dried entirely to fit for grinding. The plant materials were kept away from direct sunlight during drying to protect the phytoconstituents from degradation[29]. Then the leaves were grinded to a moderately coarse powder by using a mechanical mixture grinder. The grinding process assists the penetration of the solvent to the cellular structure of the plant tissues, which dissolves the secondary metabolites and increases the yields of extraction.

Collection of menstruum for extraction

In the present experiment, goat urine "*Ajamutra*" was used as a solvent for extraction. Goat urine was collected from two healthy female goats per day at the rate of approximately 600 mL in the morning hours in February 2018. Approximately 5 L of urine was collected and stored in an airtight glass container away from the sunlight.

Photo-activation of the solvent

The goat urine was photo activated by keeping in sunlight for about 72 h in a transparent glass

container. The photo-activation of urine was indicated by colour changes from yellowish to brown[30,31]. The bactericidal property may be raised in photo-activated urine due to the acidic nature of the photo-activated urine compared to raw urine[26]. Then, the goat urine was strained out to remove debris.

Extraction

The extraction of the leave extracts was performed by using the maceration technique. The basis of the extraction procedure was to obtain a therapeutically desirable portion and eliminate the inert insoluble material by treating a selective solvent known as the menstruum. The menstruum plays a crucial role in the qualitative and quantitative composition of the extract[32]. Moderately coarse powdered leaves of *H. sibthorpioides* and *C. papaya* were placed in the maceration chamber and macerated for seven days with raw goat urine (rGUHS and rGUCP) and photoactivated goat urine (paGUHS and paGUCP) with occasional shaking. The menstruum was strained off from the mare. The mare was pressed to recover the filtrate. The strained and expressed liquids were mixed and clarified by filtration. The amount of recovered filtrate was measured. The filtrate was evaporated to concentrate the crude extract. The concentrated crude extracts were stored in an airtight container in the refrigerator for further use.

Test microorganism

Saprophytic, rapidly growing, non-pathogenic mycobacteria; *M. smegmatis* strain was used as test organisms in the primary screening process.

Antimycobacterial susceptibility test

The antimycobacterial susceptibility test is performed by the determination of the zone of inhibition by the disc-diffusion method. It is the prescribed procedure applied in microbiology laboratories for regular antimicrobial susceptibility testing. In this method, the test microorganism's standardized 1×10^8 bacterial/mL (equivalent to 0.5 McFarland) inoculum is inoculated at agar plates. Then, about 6 mm (in diameter) filter paper discs are kept on the agar surface containing the test substance. The dishes are incubated in an incubator at a temperature of 37±1 °C. The antimicrobial substance diffuses into the agar medium and subsequently inhibits the process of germination as well as the growth of the microorganism. Finally, the diameters of the zones of inhibition are measured. The antibiogram qualitatively separates the microorganism as intermediate, susceptible, or resistant. The disc-diffusion method provides many advantages over other methods such as low cost, simplicity and the ability to test enormous antimicrobial agents[33,34].

***In vitro* antimycobacterial susceptibility assays**

Muller-Hinton agar medium

About 38 g of Muller-Hinton agar was suspended in 1 L of distilled water and then boiled to dissolve the media completely. It was sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 minutes and then allowed to cool to 50 °C. Then, 25 mL of freshly prepared medium was poured into flat bottomed sterilized Petri-plates, to give a uniform depth of approximately 4 mm. The mycobacterial strain was inoculated into the Petri-plates containing the media with the help of a sterilized inoculating loop. The plates were then incubated overnight at 37±1 °C.

Preparation of working solutions

A set of three working solutions were prepared by dissolving each of the crude extracts in sterilized distilled water in a volumetric flask to obtain concentrations of 1000, 2000, and 3000 µg/mL. The solutions were filtered through a membrane filter to remove the impurities before the test.

Preparation of inoculums

The turbidity of the inoculums equivalent to 0.5 McFarland standard was used for the susceptibility test. The standard of 0.5 McFarland was prepared by adding 0.05 mL of Barium chloride dehydrate (1.175% w/v $BaCl_2.2H_2O$) solution in 9.95 mL of Sulphuric acid (1% v/v H_2SO_4) with constant stirring to obtain a suspension containing a Barium sulphate precipitate. The suspension of bacteria was compared visually with the prepared standard[35].

Determination of the zone of inhibition

The antimycobacterial sensitivity test was carried out by the paper disc-diffusion method[27]. For determination of antimycobacterial activity, 25 mL of Muller-Hinton agar medium was poured into petri-plates and allowed to dry for 15 min. Three concentrations (1000, 2000, 3000 µg/mL) of each plant extract were prepared in 10% DMSO. About 100 µL of the inoculum was placed on the molten Muller-Hinton agar medium in petri-plates and spread throughout the plate by spread plate technique to

disperse the microorganisms homogeneously. The paper discs (6 mm diameter) were cut from Whatman filter paper No. 1 and were sterilized by autoclaving at 15 lbs (121 °C) for 15 minutes. The discs were impregnated in 10 µL above three concentrations of the rGUHS, paGUHS, rGUCP, paGUCP plant extract and kept aside for 15 minutes to dry. The extract-soaked filter paper disks were then placed on the surface uniformly seeded with test microorganisms of the Mueller-Hinton agar plates. Drug impregnated discs (Rifampicin of concentration 10 µg/mL and Berberine of concentration 25 µg/mL) were prepared by the same method. Each test plate consisted of three dilutions of the plant extract, two standards (Rifampicin and Berberine) and a disc impregnated with urine. The test plates were then incubated at 37 °C for 24 h. After 24 h of incubation, antimycobacterial activity was recorded by measuring the diameter of the zone of inhibition using a transparent ruler under the colony counter. The test was performed in triplicate to minimize the errors and the average zones of inhibition (mm) were recorded[36].

Determination of MIC

Plant extracts (rGUHS, paGUHS, rGUCP, paGUCP) were used to determine Minimum Inhibitory Concentration (MIC) using the broth microdilution method[30]. In this method, serial 5-fold dilutions of the plant extracts were prepared in the 10% DMSO. Bacterial inoculum was prepared in Mueller–Hinton broth, and the turbidity was adjusted to approximately 0.5 McFarland turbidity standards to prepare 1×10^8 bacterial/mL. the 96-well microtitre plates were used for the MIC assay. Exactly 150 µL of each plant extract was added to each well of the microplates. Exactly 50 µL of bacterial suspension was added to each well except the negative controls. Rifampicin and Berberine were used as the positive control. The 10% DMSO and plant extracts without bacterial suspension were used as the negative controls. The 96-well microtitre plates were incubated at 37±1 °C for 24 h. This procedure was repeated twice for the *M. smegmatis* strain. The MIC endpoint for the *M. smegmatis* strain was assessed by measuring absorbance at 630 nm using a microplate reader as the lowest concentration of each plant extract at which there is no absorbance was regarded as MIC[36].

Results and Discussion

In ancient India, *H. sibthorpioides* and *C. papaya* have been traditionally utilized (*Rig-Veda*) and by Chinese people to cure several infections, including bacterial and viral infections[37,38]. In the present study, we examined the *in vitro* antimycobacterial activity of the *H. sibthorpioides* and *C. papaya* leaves against the *M. smegmatis* strain. The zone of inhibition of both raw and photoactivated goat urine extracts are shown in Table 1 and Fig. 1 and 2.

Table 1 — Zone of inhibition of plant extracts against *Mycobacterium*

Name of the extracts	Zones of inhibition (mm)					
	Concentration of plant extract (µg/mL)			Goat urine	Berberine (25 µg/mL)	Rifampicin (10 µg/mL)
	1000	2000	3000			
1. rGUHS	8.33±0.47	9.66±0.43	10.66±0.47	8.41±0.47	14.74±0.47	16.41±0.47
2. phGUHS	9±0.43	10.33 ±0.57	12± 0.81			
3. rGUCP	10±1.73	11.33±0.48	12.67±0.94			
4. phGUCP	10.67±0.48	11.67±0.48	12.67±0.48			

*Values represented as mean±S.D. of replicates

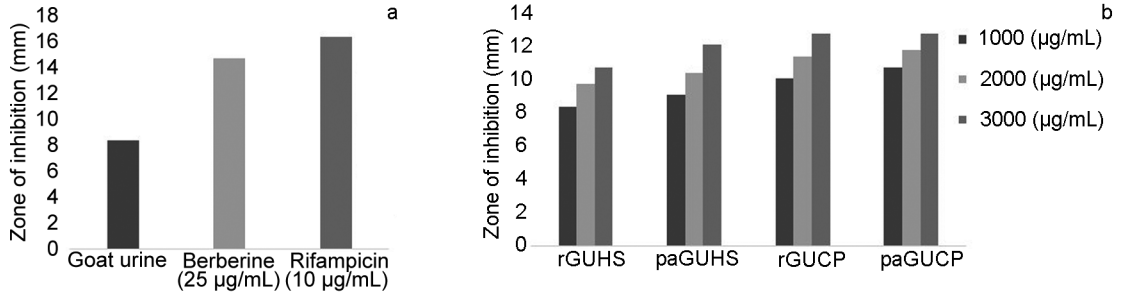

Fig. 1 — a) Zone of inhibition of Goat urine, Standard drugs against *M. smegmatis*, b) Zone of inhibition of different concentration of plant extracts against *H. smegmatis.*

From the experiment, it was found that the zone of inhibition of raw goat urine extract of *H. sibthorpioides* in the concentrations of 1000, 2000, and 3000 µg/mL were 8.33±0.47, 9.66±0.43, and 10.66±0.47 mm respectively. The zone of inhibition of photo activated goat urine extract of *H. sibthorpioides* in concentrations of 1000, 2000, and 3000 µg/mL were 9±0.81, 10.33±0.57, and 12±0.81 mm respectively. The zone of inhibition of raw goat urine extract of *C. papaya* in concentrations of 1000, 2000, and 3000 µg/mL were found to be 10±1.73, 11.33±0.48, and 12.67±0.94 mm respectively. The zone of inhibition of photo-activated goat urine extract of *C. papaya* in concentrations of 1000, 2000, and 3000 µg/mL were found to be 10.67±0.48, 11.67±0.48, and 12.67±0.48 mm respectively. The raw goat urine inhibition zone was 8.41±0.47 mm against *M. smegmatis* strain.

The MIC values obtained from plants that exhibited antibacterial activity ranged between 400±1.6 to 660±2 µg/mL against *M. smegmatis*

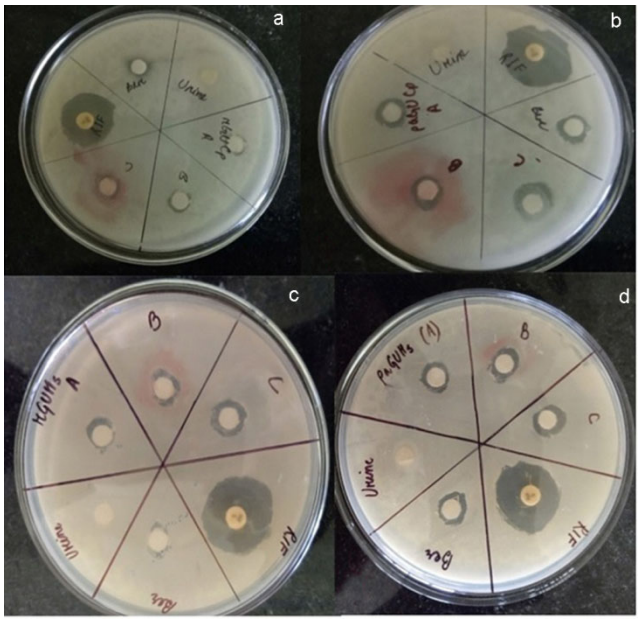

Fig. 2 — a) Antimycobacterial activity of raw goat urine extract of CP, b) Antimycobacterial activity of photoactivated goat urine extract of CP, c) Antimycobacterial activity of raw goat urine extract of HS, d) Antimycobacterial activity of photoactivated goat urine extract of HS.

(Table 2). Therefore, the highest antimicrobial activity was observed for the phGUCP extract.

It was observed that leave extracts of *H. sibthorpioides* and *C. papaya* were found to be effective in the antimycobacterial susceptibility tests. The experiment demonstrated that the extracts with photo-activated urine showed better antimycobacterial activity against *H. smegmatis* in comparison to the extracts with raw urine. Thus, both plants have a prominent antimycobacterial properties compared to the standard drugs Berberine and Rifampicin. The study also found that the antimycobacterial activity shown by both the plant materials containing goat urine as menstruum was higher than the activity demonstrated by raw goat urine. During the photo-activation process, volatile biogenic organic as well as inorganic compounds for instance acetone, methane, methanol, propanol, carbon dioxide, ammonia and secondary nitrogenous metabolic products are formed[39]. Thus, urine becomes highly acidic in comparison to fresh urine and enhances the antimycobacterial efficacy of photo-activated urine[40].

C. papaya leaves are reported to show good antibacterial activity. Romasi and co-workers reported that ethyl-acetate extract have a significant zone of inhibition in comparison with ethanol extract of leaves of *C. papaya* against *Listeria monocytogenes* and *Bacillus stearothermophililus*[41]. The researchers found that the maximum zone of inhibitions was observed for the *C. papaya* leaves at acidic pH of 4. Francis and co-workers reported that aqueous and organic (petroleum benzene) extracts of leaves and seeds of *C. papaya* demonstrated antibacterial action against the most common disease-causing bacteria namely *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia*, and *Pseudomonas aeruginosa*[38]. Kiran Peter and co-workers demonstrated that aqueous extract of *C. papaya* seeds exhibited a zone of inhibition against *S. aureus, P. aeruginosa*, and *E. coli* on increasing the concentration of seed extract[42].

Handique *et al.* reported that methanolic extract of the whole plant of *H. sibthorpioides* showed significant antibacterial activity against both gram-negative and gram-positive bacteria [*Klebsiella*

Table 2 — MIC values of plant extracts against *M. smegmatis*

Name of bacterial strain	MIC (µg/mL) of the plant extracts			
	rGUHS	phGUHS	rGUCP	phGUCP
M. smegmatis	660±2	575±1.2	540±4	400±1.6

*Values represented as mean ± S.D of replicates

pneumonia (MTCC 432) and *Staphylococcus aureus* (MTCC-96)][18]. Similarly, Mandal *et al.* showed that both methanol and aqueous extracts of *Hydrocotyle javanica* Thunb have a potential antibacterial activity against *Bacillus cereus, P. aeruginosa*, *L. monocytogenes*, and *S. aureus*[43].

In light of the different available literatures of *C. papaya* and *H. sibthorpioides* and the results of the present study, it can be concluded that these plants have effective antibacterial and antimycobacterial constituents. These phytoconstituents need to be identified and isolated to understand their activity better. Furthermore, from the study, it can be concluded that the photoactivated goat urine resulted in a synergistic effect of the extracts of *H. sibthorpioides* and *C. papaya* with the photoactivated urine and therefore improved the antimycobacterial efficacy of the plants.

Conclusion

In the present study, the antimycobacterial activity of the leaves of *H. sibthorpioides* and *C. papaya* was evaluated based on the ethnomedicinal claims of these plants as good antibacterial agents. The results showed that goat urine extract of *H. sibthorpioides* and *C. papaya* leaves have prominent activity against the *H. smegmatis* strain. The photo-activated goat urine showed significant activity in comparison to the extracts obtained using raw goat urine. The leaf extract of photo-activated goat urine extract of *C. papaya* at a 3000 µg/mL concentration showed the highest antimycobacterial activity compared to the other three extracts. In future, compounds having antimycobacterial activity can be isolated from the goat urine extracts of *H. sibthorpioides* and *C. papaya* using different analytical techniques. Furthermore, these compounds can also be studied for their effects on multidrug-resistant mycobacteria. Also, exhaustive experimentations should be carried out to understand the mechanism of antimycobacterial inhibition by *H. sibthorpioides* and *C. papaya* as well as goat urine.

Conflict of interest

The authors have no conflict of interest.

References

1 Tomar V, Nigam R, Pandey V, Singh A P, Roy D, *et al.*, Evaluation of *in vitro* anti-microbial activity of goat urine peptides, *J Anim Res*, 2018, **8**(1), 33-37.

2 Khatun Z, Bhuiyan P, Roney M S I and Rahmatullah M, Traditional knowledge on zootherapeutic practices among some folk medicinal practitioners of Bangladesh, *Am - Eurasian J Sustain Agric*, 2013, **7**(3), 155-161.

3 Lall N, Kumar V, Meyer D, Gasa N, Hamilton C, *et al.*, *In vitro* and *in vivo* antimycobacterial, hepatoprotective and immunomodulatory activity of *Euclea natalensis* and its mode of action, *J Ethnopharmacol*, 2016, **194**, 740-748.

4 Dzoyem J P, Aro AO, McGaw L J and Eloff J N, Antimycobacterial activity against different pathogens and selectivity index of fourteen medicinal plants used in southern Africa to treat tuberculosis and respiratory ailments, *S Afr J Bot*, 2016, **102**, 70-74.

5 Gautam R, Saklani A and Jachak S M, Indian medicinal plants as a source of antimycobacterial agents, *J Ethnopharmacol*, 2007, **110**(2), 200-234.

6 Brown D H, Nuttall R H, McAvoy J and Sharp D W A, Pyridine, γ-picoline, and quinoline complexes of transition metal perchlorates and tetrafluoroborates, *J Chem Soc A Inorganic, Phys Theor*, 1966, 892-896.

7 Luo X, Pires D, Aínsa J A, Gracia B, Mulhovo S, *et al.*, Antimycobacterial evaluation and preliminary phytochemical investigation of selected medicinal plants traditionally used in Mozambique, *J Ethnopharmacol*, 2011, **137**(1), 114-120.

8 Gupta R, Thakur B, Singh P, Singh H B, Sharma, V D, *et al.*, Anti-tuberculosis activity of selected medicinal plants against multi-drug resistant *Mycobacterium tuberculosis* isolates, *Indian J Med Res*, 2010, **131**(6), 809-813.

9 Laldinsanga, Sarma H, Jahan T, Goswami A K and Sharma H K, Traditional anti-malarial drugs from Serchhip and Lunglei districts of Mizoram, *Curr Trends Pharm Res*, 2019, **6**(2), 76-104.

10 Ravishankar B and Shukla V J, Indian systems of medicine: A brief profile, *Afr J Tradit Complement Altern Med*, 2007, **4**(3), 319-337.

11 Sen S and Chakraborty R, Revival, modernization and integration of Indian traditional herbal medicine in clinical practice: Importance, challenges and future, *J Tradit Complement Med*, 2017, **7**(2), 234.

12 Mahapatra A, Maheswari V, Kalia N P, Rajput V S and Khan I A, Synthesis and antitubercular activity of berberine derivatives, *Chem Nat Compd*, 2014, **50**(2), 321-325.

13 Gentry E J, Jampani H B, Keshavarz-Shokri A, Morton M D, Vander Velde D, *et al.*, Antitubercular natural products: berberine from the roots of commercial *Hydrastis canadensis* powder. Isolation of inactive 8-oxotetrahydrothalifendine, canadine, beta-hydrastine, and two new quinic acid esters, hycandinic acid esters-1 and -2, *J Nat Prod*, 1998, **61**(10), 1187-1193.

14 Wang Y, Fu H, Li Y, Jiang J and Song D, Synthesis and biological evaluation of 8-substituted berberine derivatives as novel anti-mycobacterial agents, *Acta Pharm Sin B*, 2012, **2**(6), 581-587.

15 Ozturk M, Chia J E, Hazra R, Saqib M, Maine R A, *et al.*, Evaluation of Berberine as an Adjunct to TB Treatment, *Front Immunol*, 2021, **12**, 4357.

16 Hazarika I, Mukundan G K, Sundari P S and Laloo D, Journey of *Hydrocotyle sibthorpioides* Lam.: From traditional utilization to modern therapeutics—A review, *Phytother Res*, 2021, **35**(4), 1847-1871.

17 Acharyya B K and Sharma H K, Folklore medicinal plants of Mahmora area, Sivasagar district, Assam, *Indian J Tradit Knowl*, 2004, **3**(4), 365-372.

18 Handique P J and Garg M, Evaluation of antioxidant and antimicrobial activity of a medicinal plant *Hydrocotyle sibthorpiodes* Lam., grown in NE India, *Int J Pharm Sci Res*, 2017, **8**(8), 3524-3529.

19 Ramos F, Takaishi Y, Kawazoe K, Osorio C, Duque C, *et al*., Immunosuppressive diacetylenes, ceramides and cerebrosides from *Hydrocotyle leucocephala*, *Phytochemistry*, 2006, **67**(11), 1143-1150.

20 Krishnamurthy P and Wadhwani A, Evaluation of antioxidant and antimicrobial activity of a medicinal plant *Hydrocotyle sibthorpiodes* Lam., grown in NE India, *Int J Pharm Sci Res*, 2012, **8**(8), 3524-3529.

21 Verma S, Varma R K and Singh S, Medicinal and pharmacological parts of *Carica papaya*: A review, *Indian J Drugs*, 2017, **5**(3), 88-93.

22 Sharma H K, Chhangte L and Dolui A K, Traditional medicinal plants in Mizoram, India, *Fitoterapia*, 2001, **72**(2), 146-161.

23 Jamir T T, Sharma H K and Dolui A K, Folklore medicinal plants of Nagaland, India, *Fitoterapia*, 1999, **70**(4), 395-401.

24 Murthy K R S, *Astanga Samgraha of Vagbhata*, (Chaukhambha Orientalia, Delhi), 2016.

25 Vagbhata, *Ashtanga Sangraha*, https://archive.org/details/AshtangaSangraha.

26 Hazarika S, Das S, Sarma H and Sharma H K, Application of Cow and Goat urine in traditional systems of medicines: A brief review, *Int J Pharm Biol Arch*, 2018, **9**(04), 197-203.

27 Padmanabhan P and Sujana K A, Animal products in traditional medicine from Attappady hills of Western Ghats. *Indian J Tradit Knowl*, 2008, **7**(2), 326-329.

28 Dandekar N and Shah N, Goat Milk: Boon for pulmonary Tuberculosis patients, *Int Ayurvedic Med J,* 2016, **4**(4), 584-588.

29 Haripyaree A, Guneshwor K and Damayanti M, Evaluation of antioxidant properties of some wild edible fruit extracts by cell free assays, *Elec J Environ Agric Food Chem*, 2010, **9**(2), 345-350.

30 Dhiman A, Comparison of antimicrobial, larvicidal and anthelmintic activity of *Zingiber officinale* Rose. cow urine extract, *Int J Green Pharm*, 2017, **11**(2), S280.

31 Dhiman A, Saini A and Sharma A, Comparison of antimicrobial, larvicidal and anthelmintic activity of *Curcuma aromatica* salisb. Cow urine extract, *Indian Drugs*, 2017, **54**(3), 58-61.

32 Nguta J M, Appiah-Opong R, Nyarko A K, Yeboah-Manu D, Addo P G, *et al.*, Antimycobacterial and cytotoxic activity of selected medicinal plant extracts, *J Ethnopharmacol*, 2016, **182**, 10-15.

33 Balouiri M, Sadiki M and Ibnsouda S K, Methods for *in vitro* evaluating antimicrobial activity: A review, *J Pharm Anal*, 2016, **6**(2), 71-79.

34 Jorgensen J H, Ferraro M J, Jorgensen J H and Ferraro M J, Antimicrobial susceptibility testing: A Review of general principles and contemporary practices, *Clin Infect Dis*, 2009, **49**(11), 1749-1755.

35 De Zoysa M H N, Rathnayake H, Hewawasam R P and Wijayaratne W M D G B, Determination of *in vitro* antimicrobial activity of five Sri Lankan medicinal plants against selected human pathogenic bacteria, *Int J Microbiol*, 2019, **2019**, 1-8.

36 Rathnayake H, De Zoysa M H N, Hewawasam R P and Wijayaratne W M D G B, Comparison of *in vitro* antibacterial activity of *Epaltes divaricata* and *Vetiveria zizanioides* against methicillin-resistant *Staphylococcus aureus*, *Scientifica*, 2020, **2020**, 1-8.

37 Husin F, Chan Y Y, Gan S H, Sulaiman S A and Shueb R H, The effect of *Hydrocotyle sibthorpioides* Lam. extracts on *in vitro* dengue replication, *Evid-Based Complement Altern Med*, 2015, **2015**, 1-9.

38 Francis E F and Jose V, The antibacterial effect of *Carica papaya*L. extracts and their synergistic effect with antibiotic and non-antibiotic drugs, *Br Microbiol Res J*, 2016, **16**(4), 1-11.

39 Upadhyay R K, Dwivedi P and Ahmad S, Antimicrobial activity of photo-activated cow urine against certain pathogenic bacterial strains, *Afr J Biotechnol*, 2010, **9**(4), 518-522.

40 Hu W, Murphy M R, Constable P D and Block E, Dietary cation-anion difference effects on performance and acid-base status of dairy cows postpartum, *J Dairy Sci*, 2007, **90**(7), 3367-3375.

41 Romasi E, Karina J K and Parhusip A J, Antibacterial activity of Papaya leaf extracts against pathogenic bacteria, *Makara J Technol*, 2011, **15**(2), 173-177.

42 Peter J K, Kumar Y, Pandey P and Masih H, Antibacterial activity of seed and leaf extract of *Carica papaya* var. *Pusa dwarf* Linn, *IOSR J Pharm Biol Sci*, 2014, **9**(2), 29-37.

43 Mandal M, Paul S, Uddin M R, Mondal M A, Mandal S, *et al.*, *In vitro* antibacterial potential of *Hydrocotyle javanica* Thunb, *Asian Pac J Trop Dis*, 2016, **6**(1), 54-62.