Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
NAAS Rating 2017: 3.53
JMPS 2017; 5(3): 278-283

Received: 18-03-2017
Accepted: 20-04-2017

Yumge Yomgam
a) School of Biotechnology& Biosciences, Lovely Professional University, Jalandhar, Punjab, India
b) G. B. Pant National Institute of Himalayan Environment and Sustainable Development North East Unit, Itanagar, Arunachal Pradesh, India

Rohita Sharma
School of Biotechnology& Biosciences, Lovely Professional University, Jalandhar, Punjab, India

Om Prakash Arya
GB. Pant National Institute of Himalayan Environment and Sustainable Development North East Unit, Itanagar, Arunachal Pradesh, India

Yumbi Yomgam
Arunachal Pradesh State Council for Science and Technology, Itanagar, Arunachal Pradesh, India.

Correspondence
Yumge Yomgam
a) School of Biotechnology& Biosciences, Lovely Professional University, Jalandhar, Punjab, India
b) G. B. Pant National Institute of Himalayan Environment and Sustainable Development North East Unit, Itanagar, Arunachal Pradesh, India

Phytochemical screening and antifungal activities of three medicinal plants from Arunachal Pradesh, India

Yumge Yomgam, Rohita Sharma, Om Prakash Arya and Yumbi Yomgam

Abstract
Medicinal plants have been used as sources of traditional medicine in virtually all tribal cultures. *Solanum torvum, Clerodendrum colebrookianum, and Spinlanthes acmella:* the three ethnomedicinal plants used in various ailments by tribal communities of Arunachal Pradesh were evaluated for their phytochemicals and antifungal activities against *Aspergillus niger*. Phytochemical analysis of selected plants revealed the presence of alkaloids, flavonoids, tannins, sterols, terpenoids, saponins, resins, carbohydrate in various plant parts. Ethyl acetate, Chloroform and Aqueous extracts of various parts of these plants were tested against Aspergillus *niger* by Agar well diffusion method. The obtained results showed that aqueous extract of roots of *Clerodendrum colebrookianum* and ethyl acetate extract of leaves of *Solanum torvum* were the most active; while aqueous extract of *Solanum torvum* leaves did not show any activity against *Aspergillus niger*. Presence of various phytochemicals which are associated for various activities like, antimicrobial, antioxidant, anti-inflammatory, analgesic, anticancer, antidiarrheal etc. supports the use of these plants in treatment of various ailments.

Keywords: Medicinal plant, Phytochemical, Antifungal activity, Arunachal Pradesh.

1. Introduction
The state of Arunachal Pradesh which covers a major portion of Eastern Himalaya is the largest state of north-eastern India with a geographical area of 83, 743 sq. km. spreading over 19 districts with a population of 1.255 million [1, 2]. Arunachal Pradesh is home to about 26 major tribe and 110 sub-tribes. Each district has its own composition of tribes with distinctive dialects, custom, traditional beliefs and cultural diversity. In Arunachal Pradesh there is 5000 species of angiosperms, of which 500 species has been reported so far. These plants have been used by locals to cure various ailments and considered as potential source of economy to the state [3, 4]. Medicinal plants have been used as sources of traditional medicine in virtually all tribal cultures. Each tribe have its own traditional believe and histories of treating ailments through preparing herbal extracts from the surrounding and incantation of local hymns [5]. Over the centuries the local population of Arunachal Pradesh have gain an abundant knowledge on the utility of various plant species and forest commodity [6].
The tribal communities are mostly forest dweller who are engaged in agriculture, fishing, hunting, animal husbandry etc and increases the chance of skin infection due to various fungal and bacterial infections. Skin disease accounts for 34% of all occupational ailments [6, 7]. Over the past decade the incidence of fungal infection has risen substantially, which are higher in case of immunocompromised patients. Inappropriate and extensive use of immunosuppressant and antifungal drugs has increases the frequency of opportunistic fungal infection. *Aspergillu*s spp., *Candida* albicans, *Malassezia* spp. and *Fusarium Trichosporon* are some of the most common opportunistic fungi causing life threatening invasive disease [8, 9]. A wide range of disorders including allergy, colonization and invasive disease is caused by Aspergillus species in immunocompetent and immunocompromised individual. It is estimated that the mortality due to fungal infection is around 1,350,000 patients per year across the world [10]. Antimicrobial activity is the ability of a substance to inhibit growth or kill microorganism. Medicinal plants have antimicrobial activity, as they produce wide range of bioactive molecules which kills or inhibit the growth of microorganism such as bacteria, fungi, or protozoa [11, 12]. A vast majority of plants have not been adequately evaluated, even though thousands of plant varieties from different parts of India have been tested for antimicrobial activities [13].

Three ethnomedicinal plants viz *Spilanthes acmella*, *Clerodendrum colebrookianum* and *Solanum torvum* (Fig.1) from Arunachal Pradesh, traditionally used in various ailments (Table 1) were undertaken to investigate the screening of secondary metabolites and antifungal activities against *Aspergillus niger*.

Fig 1: Ethnomedicinal plants under study (a) *Clerodendrum colebrookianum*, (b) *Spilanthes acmella* (c) *Solanum torvum* from Arunachal Pradesh

Table 1: The ethnobotanical information of selected medicinal plants species

Species Name	Family	Local name	Plant parts used	Traditional Uses
Clerodendrum colebrookianum	Lamiaceae	Oen-tapen (*Galo*), ongin (*Nyishi*)	Tender leaves,	Blood pressure [13]; stomach trouble, malarial and bronchitis treatment [14]
Solanum torvum	Solanaceae	Baka (*Galo*), Byako (*Adi*), Byakta (*Nyishi*), Sathi Byako (*Apatani*)	Fruits, seed and roots	Infection in gums and tootache [15]; stomachache, high blood pressure [15] fever, cough, toothache [16] malaria and stomach pain [17]
Spilanthes acmella	Asteraceae	Marsha(*Galo*), Marsang (*Adi*)	Leaves and young twigs	Stop bleeding, skin infections and gastric, fish poison [12]

* Words in parenthesis in the column 3 is the name of ethnic communities of Arunachal Pradesh

2. Materials & Methods

2.1. Plant collection and identification

The leaves, stem, bark and roots of *Spilanthes acmella*, *Clerodendrum colebrookianum* and *Solanum torvum* were collected from Itanagar and Diomukh area of Arunachal Pradesh, India. The plant was identified at BSI, SFRI and State Medicinal Plant Board (SMPB) Arunachal Pradesh.

2.2 Sample preparation

The plant material was sorted and cleaned in running water and the plant parts were air-dried in the shade at room temperature till constant weight was attained. The dried parts of plants were chopped into tiny pieces and then grinded with mortar pestle into powder form, sieved and packaged into air tight containers for further experimental work.

2.3 *Plant Extracts Preparation*

The extraction process was carried out with three different solvents viz. ethyl acetate, chloroform, and water. Plant samples were extracted with various solvents using Rotary shaker and Soxhlet extraction method as shown in Table 2. For extraction of plant material in water, powdered plant parts were kept in a rotary shaker for 3 days; while with other solvents extraction was performed using Soxhlet extraction method. All the extracts were first filtered through Whatman no. 1 filter paper to remove the debris. All the extracts were evaporated using rotary vacuum evaporator and percentage yield was thus calculated. The dried plant extracts were then solubilised in 2 ml of solvents and 2ml of 2% DMSO in normal saline was added to it and kept in centrifuge tubes. The centrifuge tubes levelled, sealed and kept at 4^0C in the refrigerator for further screening of phytochemical and antifungal activity of these plant extract against *Aspergillus niger*.

Table 2: Complete chart of various parts of plant extract

Species name	Plant parts	Solvent	Extraction method	Initial amount
Clerodendrum colebrookianum	Leaves	Ethyl acetate	Soxhlet and rotary evaporator	4g
Clerodendrum colebrookianum	Leaves	Chloroform	Soxhlet and rotary evaporator	5g
Clerodendrum colebrookianum	Roots	Water	Rotary shaker and rotary evaporator	5g
Solanum torvum	Leaves	Ethyl acetate	Soxhlet and rotary evaporator	4g
Solanum torvum	Leaves	Water	Rotary shaker and rotary evaporator	5g
Solanum torvum	Stem	Ethyl acetate + Water	Rotary shaker and rotary evaporator	4g
Spilanthes acmella	Whole plants	Ethyl acetate	Soxhlet and rotary evaporator	3g
Spilanthes acmella	Whole plants	Water	Rotary shaker and rotary evaporator	3g

2.4 Phytochemical analysis
Chemical tests for the screening of different phytoconstituents in *Spilanthes acmella*, *Clerodendrum colebrookianum* and *Solanum torvum* were performed following the standard methods [18-20]

Test for alkaloids
1ml of extract mixed with some drops of dilute HCl and then filtered out. From this 1ml of extract is taken and 1 ml of Meyers reagent was added. Creamy white precipitation indicates the presence of alkaloids.

a) Test for saponins
In 1 ml of extract, 2ml of distilled water added and shake continuously for 10 minutes. Formation of foam on the mixer surface after for 20 minutes confirms the existence of saponins.

b) Test for flavonoid
i. Alkaline reagent test
Few drops of NaoH solution blended with 2 ml of extract solution. Development of intense yellow colour confirms the presence of flavonoid.

ii. Lead acetate test
2 ml of extract put together with few drops of 10 % lead acetate, presence of yellow colour confines the existence of flavonoids

c) Test for sterols
i. Sulphuric acid test
Extract from plant mixed with sulphuric acid along with ethanol gives rise to blue green colouration suggests the presence of sterol

d) Test for tannins
1 ml of extract combined 2ml of 5% ferric chloride. Dark brownish green colour shows the presence of both phenols as well as tannins.

e) Test for Terpenoids
1ml of plant extract and 2ml of chloroform blended thoroughly, and then 3 ml of sulphuric acid added to it leads to formation of layer. Reddish brown colouration indicates the presence of terpenoids.

f) Test for carbohydrate
2 mg of extract added to 1 ml of distilled water. The mixture is then filtered to remove the debris.
i. Benedict's test
0.5 ml of filtrate from the above mixture was combined with 0.5 ml of Benedict's reagent. The solution was then heated in water bath for 2 minutes. Characteristic precipitate formation determines the occurrences of carbohydrate.

g) Test for resins
i. Acetone water test
Small amount of water and 1 ml of acetone was added to extract. The mixer is shaken well. Appearance of turbidity in the solution indicates the presence of resins in the extract.

2.5 Test microorganism and maintenance
In the present investigation, *Aspergillus niger (MTCC 501)* was collected from Lovely Professional University (LPU) laboratory, Punjab.

2.6 Antifungal activity
i. Fungal culture
In the present investigation, *Aspergillus niger* was cultured in potato dextrose agar by spread plate method in three plates and streak plate method in other two plates and then incubated at 28^0 C for 7 days. On the second day, inoculums appeared as creamy coloured colonies in the petri dishes. On the seventh day mature blackish dark green numerous spore colonies as dots was observed. Sub culturing was done by both spread plate and streak plate method once culture reached confluence. Inoculation and harvesting was prepared as described by Yazdani *et al*. [21] with few modifications. On the 7^{th} day conidia from matured colonies was harvested by flooding (0.5% v/v) of 5ml tween 20 aseptically inside the laminar flow hood. Then the solution is filtered through Whatman No.1 filter paper. To check the viability and verify the absence of contamination two plates were inoculated from the above prepared solution and incubated for seven days. On seventh day mature dark spore conidia without any contamination was obtained. The suspended conidia spores kept at 4^0 C in the refrigerator for further use.

ii. Media & plate preparation
Every glassware and plastic ware along with nutrient agar was sterilized in autoclave and hot air oven. The media used for antifungal activity were Potato dextrose agars. 20 ml of pre autoclaved Potato dextrose agar (MHA) was poured into pre sterilized Petri plates. These Petri plates were led to solidify at room temperature.

iii. Agar well diffusion method
The agar well diffusion was chosen as it is globally accepted testing method for microbial susceptibility [23]. Antifungal activities of all the plant extract were tested using agar well diffusion method according to Varaprasad *et al*. [24] with slight modification. After solidifying of the media, 50 µl of conidia (*A.niger)* suspended in 0.5% v/v tween 20 was taken in a 100 µl pipette then spread thoroughly using a glass rod. Then the wells were punched using autoclaved cork borer. Plant extract were placed in the well carefully using a micro pipette. The plates were then sealed with parafilm and subjected to incubation in upright position at 28^0 C for initiation of fungal growth. To ensure the sterility all the work was carried out in laminar flow hood. After incubation, the diameter of zone of clearance of inhibition produced around each well were measured in cm and compared with standard antifungal drugs.

3. Results
3.1. Extract yield
The yield of different parts of the plants under study is given below in the table (Table 3). Ethyl acetate gives a higher yield for the leaves of Clerodendrum colebrookianum and Solanum torvum amongst other solvents. Water have different yield for different plants which also varies according to their plant parts. Whole plant of *Spilanthes acmella* have highest yield (22.4%) followed by Stem of *Solanum torvum* (17%) and whole plant of *Spilanthes acmella* (14.7%) amongst all the plant parts.

Table 3: Extract yield of different parts of three selected medicinal plants

Plant species	Plant Parts	Solvent	Mode of extraction	Final amount	Yield
Clerodendrum colebrookianum	Leaves	Ethyl acetate	Soxhlet and rotary evaporator	0.55g	13.75%
Clerodendrum colebrookianum	Leaves	Chloroform	Soxhlet and rotary evaporator	0.47g	9.4%
Clerodendrum colebrookianum	Roots	Water	Rotary shaker and rotary evaporator	0.60g	12%
Solanum torvum	Leaves	Ethyl acetate	Soxhlet and rotary evaporator	0.55g	13.75%
Solanum torvum	Leaves	Water	Rotary shaker and rotary evaporator	0.50g	10%
Solanum torvum	Stem	Ethyl acetate + Water	Rotary shaker and rotary evaporator	0.68g	17%
Spilanthes acmella	Whole plants	Ethyl acetate	Soxhlet and rotary evaporator	0.44g	14.7%
Spilanthes acmella	Whole plants	Water	Rotary shaker and rotary evaporator	0.67g	22.4%

3.2. Phytochemical analysis

Phytochemical analysis of selected plants revealed presence of various phytochemicals Table 4. So*lanum torvum* showed the presence of tannin, resins, saponins, terpenoids, carbohydrate and alkaloids, however flavonoids and sterols were absent from the *Solanum torvum* extracts. Except terpenoids all other tested secondary metabolites was present in the *Clerodendrum colebrookianum*. *Spilanthes oleracea* reported the absence of sterols, saponins and flavonoids, whereas alkaloids, tannins, resins, carbohydrate and terpenoids were present. The presence or absence of particular phytochemical also depends on the nature/abilities of the solvent used.

Table 4: Table for phytochemical analysis for plant extract

Plant species ⟹	***Spilanthes acmella***		***Solanum torvum***			***Clerodendrum colebrookianum***		
Solvents ⟹ Phyto-chemicals ⇩	Ethyl acetate (Whole plant)	Water (Whole plant)	Ethyl acetate (leaves)	Water (Leaves)	Ethyl acetate + Water (Stem)	Ethyl acetate (Leaves)	Chloroform (Leaves)	Water (Root)
Alkaloids	-	+	+	+	-	-	-	+
Flavanoid	-	-	-	-	-	+	-	-
Saponins	-	-	-	+	-	-	-	+
Sterols	-	-	-	-	-	+	+	-
Tannin	+	+	+	+	-	+	-	-
Tarpenoid	-	+	-	-	+	+	-	-
Resins	+	+	+	+	+	+	+	+
Carbohydrate	+	-	+	-	-	+	+	+

3.3. Antifungal activity

The inhibitory effect of various plant extract on mycelium growth of *Aspergillus niger* varies as shown in Table 5**.**The highest inhibitory zone (2.475 cm) was shown by *Clerodendrum colebrookianum* in water. *Solanum torvum* leaf extract in ethyl acetate and *Spilanthes acmella* in ethyl acetate exerted an inhibition of 1.50 cm and 1.575 cm respectively. *Clerodendrum colebrookianum* leaf extract in chloroform also showed significant antifungal activity of 1cm i.e. diameter of zone of inhibition. Others showed medium activities against the mycelium growth that is between 0.6 cm to 0.75 cm. *Solanum torvum* extracted in water did not show any antifungal activity against *Aspergillus niger*. Three controls were used for this study: - Itraconazole, voriconazole and amphotericin B. Itraconazole showed the highest inhibitory activity of 2.38 cm against the *Aspergillus niger*. A diameter of 2.3 cm was given by voriconazole around the wells, while amphotericin B showed 1.70 cm of inhibition zone. The Diameter of zone of inhibition by different plant extract exhibited by different plant extract also depends on solvents.

Table 5: Diameter of zone of inhibition by different plant extract

Plant parts →	**Clerodendrum colebrookianum**			**Solanum torvum**			**Splanthes acmella whole plant**	
	Leaves	**Leaves**	**Roots**	**Leaves**	**leaves**	**Stem**		
Solvents	Ethyl acetate	Chloroform	Water	Ethyl acetate	Water	Ethyl acetate + Water	Ethyl acetate	Water
Zone of inhibition (cm)	0.675	1	2.475	1.575	-ve	0.75	o.6	1.5

4. Discussion

Alkaloids are associated with properties such as antibacterial, analgesic and antipasmodic. Tannins are found to be astringent in nature, antimicrobial, anticancer, hasten the healing of wounds, antitumor and inflamed mucous membrane. Antioxidant, anticancer and antimutagenic characteristics are found in saponins. Flavonoids are bestowed with activities such as antioxidant, cytostatic, antiallergic, analgesic, anti-inflammatory, antidiarrheal and antimicrobial. Whereas, experiments on phenols showed various activities like antioxidant, reduce blood pressure, decrease viscosity of blood, diuretic and chloeretic functions. Experiments on sterols confirm anti-inflammatory and analgesic activities. Carbohydrate accounts for nutritive value. Terpenoids play a vital role in defence system of plants and also use in manufacturing sustainable pesticide and abiotic stress protection [25, 26]. Previously, researcher have reported the presence of alkaloids, flavonoids, saponins, steroids, tannins, tarpenoid, glycosides and phenolic compounds in the leaves and seeds of S*olanum torvum* [27, 28]. Researchers have reported presence of alkaloids, carbohydrates, tannins, steroids, carotenoids, glycosides, flavonoids, terpenoids, phlobatannins, fats and fixed oils, sesquiterpenes and amino acids in different extracts of Spilanthes acmella plant parts [29, 30 31]. Experiments have reported the presence of glycosides, terpenoids, tannins, saponins, steroids, phenols, flavonoids, alkaloids, carotenoids, carbohydrate and proteins in *Clerodendrum colebrookianum* [26, 32]. This study was the first of its kind from Arunachal Pradesh for these three plants, results may be vary from earlier researchers' who worked on

the same plants, because of different habitat of these plants and use of different solvents.

5. Conclusion

The present study showed that different plant parts show significant antifungal activities against *Aspergillus niger*. The different plant parts also revealed presence of different phyto-chemicals which are associated for various activities like, antimicrobial, anti-oxidant, anti-inflammatory, analgesic, anticancer, antidiarrheal etc. supports the use of these plants in treatment of various ailments by ethnic communities of Arunachal Pradesh. Further studies should be performed for the isolation and characterization of bioactive compounds so that selected plant species may be use as potent drugs.

6. Acknowledgements

Authors are thankful to Head of the Department, School of Biotechnology& Biosciences, Lovely Professional University, Punjab for providing the support and facilities. Authors are also thankful to the local communities of the Doimukh and Itanagar for their support and help in collection of plant material.

7. References

1. Doley B, Gajurel PR, Rethy P, Buragohain R. Uses of trees as medicine by the ethnic communities of Arunachal Pradesh, India, J. Med. Plant Res. 2011;8: 857-863.
2. http://www.census2011.co.in/census/state/districtlist/arunachal+pradesh.html
3. Haridarsan K, Anupam S, Bhuyan LR, Bisht NS, Medicinal Plants Sector in Arunachal Pradesh: An Overview. Indian Forester 2003; 129:37-47.
4. Gupta M, Manisha K, Grover R. Effect of various media types on the rate of growth of *Aspergillus niger*. Indian journal of fundamental and applied life science 2012; 2(2):141-14.
5. Kohli YP, Non-traditional foods and ethnobotanical plants of Lower Subansiri district, Arunachal Pradesh. Arun. Fo. News. 2001; 19(2):169-171.
6. Spiewak R. Occupational skin diseases among farmers. In: Zagorski, J. (Ed.), Occupational and Para-occupational diseases in Agriculture. Institute of Agriculture Medicine, Lublin, Poland. 2000: 142-152.
7. Namsa ND, Tag H, Mandal M, Kalita P, Das AK. An ethnobotanical study of traditional anti-inflammatory plants used by the Lohit community of Arunachal Pradesh, India. Journal of Ethnopharmacology 2009; 125:234-245.
8. Ramana KV, Kandi S, Bharatkumar V P, Sharada CHV, Rao R, Mani R *et al.* American Journal of Infectious Diseases and Microbiology. 2013; 1(4):64-69.
9. Singh T, Kashyab AK, Ahluwalia G, Chinna D, Sidhu SS. Epidemiology of fungal infection in critical care setting of tertiary care teaching hospital in North India: A prospective of surveillance study. JClinSci Res 2014; 3:14-25.
10. Rudramurthy SM, Singh G, Hallur V, Verma S, Chakrabarti A. High fungal spore burden with predominance of Aspergillus in hospital air of tertiary care hospital in Chandigarh. Brief communications 2016; 34(4):529-532.
11. Ramaswamy S, Charles MA. Antibacterial effect of volatile components of selected medicinal plants against human pathogens. Asian Journal Microbial Biotech Environment 2009; 6:209-210.
12. Namsa ND, Mandal M, Tangjang S, Mandal SC. Ethnobotany of Monpa ethnic group at Arunachal Pradesh, India. Journal of ethnobiology and ethnomedicine 2011; 7:31 DOI: 10.1186/1746-4269-7-31.
13. Mahesh B, Satish S. Antimicrobial activity of some important medicinal plant against plant and human pathogens. World journal of agricultural science 2008; 4:839-843.
14. Srivastava RC, Adi community. Traditional knowledge of Adi tribe of Arunachal Pradesh on plants. Indian journal of traditional knowledge 2009; 8(2):146-153.
15. Murtem G, Chaudhry P. An ethnobotanical study of medicinal plants used by the tribes in Upper Subansiri district of Arunachal Pradesh, india. American journal of ethnomedicine 2016; 3(3):35-39.
16. Baruah S, Borthakur SK, Gogoi P, Ahmed M. Ethnomedicinal plants used by Adi-Minyong tribe of Aunachal Pradesh, eastern Himalaya. Indian journal of Natural product and Resources. 2013; 4(3):278-282.
17. Arya OP, Pandey A, Samal PK. Ethnobotany and Nutritional Importance of four selected Medicinal Plants from Eastern Himalaya, Arunachal Pradesh. Journal of Medicinal plants studies 2017; 5(1):45-49.
18. Das AK, Tag H. Ethnomedicinal studies of the Khamti tribe of Arunachal Pradesh. Indian journal of traditional knowledge 2006; 5(3):317-322.
19. Khan FA, Hussain I, Farooq S, Ahmad M, Arif M, Rehman IU *et al.* Phytochemical screening of some Pakistanian medicinal plants. Middle-East journal of scientific research 2011; 8(3):575-578.
20. Kumar RS, Venkateswar C, Samuel G, Rao GS. Phytochemical screening of some compounds from plant leafs extracts of Holoptelea integrifolia (Planch.) and Celestrusemarginata (Grah.) used by Gondu tribes at adilabad district, Andhra Pradesh, India. International journal of engineering science invention 2013; 2(8):65-70.
21. Zohra SF, Meriem B, Samira S, Alsayadi MMS. Phytochemical screening and identification of some compound from Mellow. Scholars research library 2012; 2(4):512-516.
22. Yazdani D, Zainal Abidin MA, Tan YH, Kamaruzaman S, Jaganath IB. Screening of phytochemical from ethnomedicinal plants in Malaysia for use against toxigenic Aspergillus flavus. Journal of Medicinal Plants Research. 2012; 6(42):5464-5468.
23. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests, 7th ed. Approved standard. M2-A7. NCCLS, Wayne, Pennsylvania, 2000.
24. Varaprasad B, Katikala PK, Naidu KC, Penumajji S. Antifungal activity of selected lants extracts against phytopathogenic fungi *Aspergillus niger* F2723. Indian journal of Science and technology. 2009; 2(4):87-90.
25. Sumathy R, Vijayalakshmi M, Deecaraman M, Sankaranarayanan S, Bama P, Ramachandran J *et al.* Screening of secondary metabolites, antioxidant and antimicrobial activity from petals of Moringaoleifera. World journal of pharmacy and pharmaceutical science 2014; 3(6):1829-1843.
26. Thangi J, Ashwini HA, Singh KG. Qualitative and quantitative phytochemical analysis of ethnomedicinal folklore plant- *Clerodendrum colebrookianum*. Journal of

global biosciences 2016; 5(1):3559-3566.
27. Khatoon N, Jain P, Choudhary AK. Phytochemical studies on seed and leaf extract of *Solanum torvum* SW. Indo American journal of Pharmaceutical research. 2015; 5(5):1649-1659.
28. Yousaf Z, Wang Y, Baydoun E. Phytochemistry and pharmacological studies on *Solanum torvum* Swartz. Journal of applied pharmaceutical science 2013; 3(4):152-160.
29. Yadav R. Phytochemical screening of Spilathesacmella plant parts. International journal of pharmaceutical erudition. 2012; 1(4):44-28.
30. Soni S, Dhurwe V, Khan S, Soni UN. Phyto-chemical investigations from leaves and flower of Spilanthes acmella murr. International journal of science and research methodology 2016; 4(1):70-76.
31. Kavya MD, Pramod VP. Phytochemical and pharmacognostic investigations of Spilanthesacmella MURR. World journal of pharmacy and pharmaceutical sciences. 2015; 4(11):978-988.
32. Majaw S, Moirangthem J. Qualitative and quantitative analysis of *Clerodendrum colebrookianum* Walp. Leaves and Zingiber cassumunar Roxb. Rhizomes. Ethnobotanical leaflets 2009; 13:578-589.