Research Article

Screening of Phytochemicals, Antioxidant and Antimicrobial Activity of Some Tea Garden Weeds of Tinsukia, Assam.

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ABSTRACT

The objective of this study was to evaluate the phytochemical constituents, antioxidant and antimicrobial activity of five medicinal plants - *Mikania micrantha*, *Cynodon dactylon*, *Borreria hispida*, *Oxalis corniculata* and *Oxalis acetosella.* The extract was prepared using methanol as solvents. Total phenolic content and antioxidant activity were determined spectrophotometrically. All the plant extracts were tested for antimicrobial efficacy against five Gram Positive bacterial strains viz, *Bacillus subtilis* (MTCC 441), *Bacillus cereus* (MTCC 8750), *Staphylococcus aureus* (MTCC 3160), *Staphylococcus epidermis* (MTCC 3615) and *Proteus vulgaris* (MTCC 744); two Gram Negative strains viz, *Escherichia coli* (MTCC 443), *Enterococcus faecalis* (MTCC 439) and two fungal strains viz- *Candida albicans* (MTCC 3017) and *Penicillium chrysogenum* (MTCC 947). Good amount of total phenolic content and antioxidant activity were observed in the entire sample. All the extracts demonstrated moderate antibacterial activity against certain tested pathogens, while inactive against *P. chrysogenum.* This study scientifically supports the usage of these plants as a remedy for various ailments in traditional medicine.

Keywords: Antioxidant and Antimicrobial activity, Phytochemicals, Total phenolic content.

INTRODUCTION

In India, throughout its long history, has accumulated a rich body of empirical knowledge of the use of medicinal plants for the treatment of various ailments. There exists a plethora of knowledge about herbal drugs in our ancient literature of Ayurvedic and Unani medicine. The Charaka Samhita mentioned the use of over 2000 herbs for medicinal purpose.[1] Herbs have provided us some of the very important lifesaving drugs used in the armamentarium of modern medicine.[2] World Health Organization has indeed recognized medicinal plants as the best source for obtaining a variety of synthetic drugs.[3] Chemical studies of Indian medicinal plants play an important role for the development of new drugs of natural origin. In recent years, phytochemicals have been extensively investigated as a source of medicinal agents.[4] The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants.[5] The selected plants of the present study *Mikania micrantha* H.B.K. (Asteraceae), *Cynodon dactylon* (L) Pers. (Poaceae), *Borreria hispida (L.) Schum.* (Rubiaceae), *Oxalis corniculata Linn.* (Oxiladaceae) and *Oxalis acetosella Linn.* (Oxiladaceae) were commonly used in various ailments by different ethnic groups of Assam. All these plants were extensively found in the tea gardens of Assam as weed. Ethomedicinally *M. micrantha* is used to treat fever, rheumatism, influenza and respiratory diseases; the juice of *C. dactylon* was used to treat hysteria, epilepsy, for purifying the blood, diarrhoea, gonorrhoea; *O. corniculata* and *O. acetocella* traditionally used in anaemia, dysentery, diarrhoea, skin diseases; the vapour of *B. hispida* is inhaled to kill tooth-worms; jaundice**,** decoction of the herb used to relieve headache. Hence the present study is focused to evaluate the phytochemical component, antioxidant and antimicrobial activity of these plants.

MATERIALS AND METHODS

Sample collection

Plants were collected from tea garden areas of Tinsukia, Assam. The materials were shade dried and grounded to fine powder using electric grinder.

Sample extraction

Samples were macerated with methanol for 48 hours and filtered through Whatman No 1 filter paper. The filtrate was then evaporated at a constant temperature (60°C) until a semi dried powder/sticky mass of crude extract was obtained. The crude extract was dissolved in Dimethyl sulphoxide (DMSO) as neutral solvent to make final concentration for biochemical analysis.

Experimental

Following methods were used for the phytochemical analysis, antioxidant & antimicrobial activity of the selected plants-

Phytochemical analysis, total phenol content (TPC) and total flavonoid content (TFC), antioxidant activity

The qualitative phytochemical analysis was performed following the standard laboratory methods described by Edeoga et al.[6]; Aja et al.[7] and Ajayi et al.[8] Quantitative estimation of TPC was done by the method described by Malik and Singh[9] and TFC by the method described by

Mervat and Hanan.[10] Antioxidant activity study was performed using DPPH and ABTS radical scavenging method as described by Anti-Stanojevic et al.[11] and Re et al. [12] respectively.

Antimicrobial activity study

The antimicrobial test was carried out by agar well diffusion method described by Nair et al.[13] using 6mm borer. The activity was determined by measuring the diameter of zone of inhibition (ZOI) exhibited by the extract.

Selected strains for antimicrobial study

Five Gram Positive bacterial strains viz, *Bacillus subtilis* (MTCC 441), *Bacillus cereus* (MTCC 8750), *Staphylococcus aureus* (MTCC 3160), *Staphylococcus epidermis* (MTCC 3615) and *Proteus vulgaris* (MTCC 744); two Gram Negative strains viz, *Escherichia coli* (MTCC 443), *Enterococcus faecalis* (MTCC 439) and two fungal strains viz- *Candida albicans* (MTCC 3017) and *Penicillium chrysogenum* (MTCC 947) were used in the study. Strains were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The reference of bacterial strains were maintained on nutrient agar slants and fungal strains on PDA slants and stored in freeze. Strains were regularly subcultured using nutrient broth for bacterial strains and PDB for fungal strains.

Standard antibiotics

Standard antibiotics viz, Chloramphenicol (C) 30mcg, Clotrimazole (CC) 10mcg, Ampicillin (AP) 10mcg, were taken for bacterial strains and for fungi, Nystatin (NS) 50mcg, Clotrimazole (CC) 10mcg, Ampicillin (AP) 10mcg, were employed for comparison of ZOI with sample.

RESULTS AND DISCUSSION

The phytochemical constituents of the plants are in the Table 1. From the table, presence of tannin, phenol, flavonoid, glycoside, cardiac glycoside were recorded in all the samples. Alkaloids were found in *O. corniculata* and *O. acetocella*; terpenoid in *C. dactylon* and *B. hispida*; carotenoid in *M. micrantha, O. acetocella* and *O. corniculata*; saponin in *M. Micrantha, C. dactylon* and *O. corniculata*. Except *B. hispida* none of the plants had steroid in their methanolic extract. Study conducted by Kaleeswaran et al.[14] in ethanolic extract of *C. dactylon* shows absence of alkaloid, steroid and triterpenoid and presence of phenol, tannin which support our results; on the other hand, they shows absence of flavonoid, glycoside, saponin which is not in line with us. Rahee and Mallik[15] reported the presence of steroid, alkaloid, glycoside and tannin and absence of flavonoid and saponin in methanolic extract of *B. hispida*. Raghavendra et al.[16] shows the presence of glycoside, phytosterols, phenolic compound, tannins and flavonoids in methanolic and ethanolic extract of *O. corniculata*, but absent in petroleum ether, benzene and chloroform except flavonoid in chloroform extract. These variations of presence and absence of phytoconstituents in the present study and earlier study may happen because of habitat differences of the plants which play important role in production of secondary plant metabolite.

Table 1: Qualitative analysis of phytochemicals in the plants

Phytochemicals	*Mikania micrantha*	*Cynodon dactylon*	*Borraria hispida*	*Oxalis acetosella*	*Oxalis corniculata*
Tannin	+	+	+	+	+
Alkaloid	-	-	-	+	+
Phenol	+	+	+	+	+
Flavonoid	+	+	+	+	+
Terpenoid	-	+	+		
Steroid	-	-	+	-	-
Glycoside	+	+	+	+	+
Cardiac glycoside	+	+	+	+	+
Carotenoid	+	-	-	+	+
Free anthraquinone	-	-	-	-	-
Saponin	+	+	-	+	-

+ indicates presence of constituents and – indicate absence of constituents

Table 2 presents the TPC and TFC of methanolic extract of the plants. TPC in terms of catechol equivalent (the standard curve equation y = 0.318x) were between 1.62 to 3.34 mg/gm dry material and TFC in terms of quercetin equivalent (the standard curve equation y = 0.347x) were between 1.34 to 2.16 mg/gm dry material. The radical scavenging activity of different plant extracts are presented in Table 3. The study revealed that methanolic extract of the plants showed significant DPPH and ABTS radical scavenging activity, though the values are less than ascorbic acid. Shajiselvin & Muthu[17] showed that *B. hispida* has 4.8 + 0.073 mg/g dry material TPC in terms of catechol equivalent and 84.75+0.024% antioxidant activity. Methanolic extract of *O. corniculata* showed

potent antioxidant activity.[18] Ethanolic extract of *C. dactylon* shows 4.029 mg/g dry material TPC in terms of tannic acid equivalent, 0.17 mg/g dry material TFC in terms of quercetin equivalent and 78.06% (in mg/ml) DPPH and 76.63% (in 1.5 mg/ml) ABTS.[19] Hui et al.[20] reported that *M. micrantha* has good amount of TPC, TFC and antioxidant activity.

Table 2: Total Phenolic and total flavonoid content of the plants

Samples	Phenol (mg catechol equivalent/gm dry material	Flavonoid (mg quercetin equivalent/gm dry material
	Methanol extract	Methanol extract
Mikania micrantha	3.34± 0.02	2.07± 0.03
Cynodon dactylon	1.62± 0.34	1.34± 0.11
Borraria hispida	3.19± 0.22	2.16± 0.19
Oxalis corniculata	2.22± 0.08	1.62± 0.29
Oxalis acetosella	1.95±0.09	2.07±0.01

Table 3: Antioxidant activities of the plants

Sample	Antioxidant activity (% inhibition in mg/ml)	
	DPPH radical scavenging activity	ABTS radical scavenging activity
	Methanol extract	Methanol extract
Mikania micrantha	63.57± 0.13	75.20± 0.34
Cynodon dactylon	23.04± 0.33	73.06±0.03
Borraria hispida	44.42± 0.23	74.40± 0.01
Oxalis corniculata	17.47± 0.43	74.60± 0.00
Oxalis acetosella	65.05±0.21	73.33±0.00
Ascorbic acid	88.20±0.10	83.00±0.00

This study revealed that methanolic extract of the plant comprise effective potential source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. It is also observed that total phenol and flavonoid content and antioxidant activity of the plants varies among them depending on habitat condition of the plants.

Table 4 and table 5 presents the zone of inhibition of methanol extract against certain bacterial and fungal strains in comparison to certain standard antibiotics. The results of the present study are encouraging as all the plants tested possessed antimicrobial activity against two or more tested microbes, while all the plant extracts were completely resistant to *P. chrysogenum.* No single plant was found to be equally effective against all the microbes tested, which responded in a varied manner. Similar studies of these plants have also been conducted by several other workers[16,21,14] . The results of a study has shown that the zone of inhibition of the methanolic and ethanolic extract of *O. corniculata* were 16.87mm and 13.39mm respectively for *S. aureus* and 1.00mm and 8.10mm respectively for *E. coli* [16]. Muthu et al. [21] revealed that methanolic extract of *Borreria hispida* was found maximum antibacterial activity than petroleum ether and ethyl acetate extracts; methanolic extract shows 15mm, 18mm, 16mm and 15mm zone of inhibition against *B. subtilis, E. coli, P. aruginosa* and *S. aureus* respectively in 100mg/ml concentration; Chethan et al.[22] reported no activity against *B. subtilis, E. coli, B. cereus* and 12 mm zone of inhibition against *S. aureus*. Kaleeswaran et al. [14] reported *C. dactylon* shows 15mm, 14mm, 11mm and 15mm zone of inhibition against *B. subtilis, E. coli, P. aruginosa* and *S. aureus* respectively. Thus the variation observed between present and earlier study could be attributed to the concentration of the sample, method of extraction and habitat condition of the plants.

The present study revealed that all the tested plants are good source of natural phenolic compounds and their possible application against microorganisms such as *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus* etc. Further studies may lead to their use as natural antioxidants and as safe alternatives to synthetic antimicrobial drugs.

Table 4: Antibacterial activity of the plants

Test sample	Diameter of inhibition of zone (mm)						
	B.subtilis	*B.cereus*	*S.aureus*	*S.epidermis*	*P.vulgaris*	*E.faecalis*	*E.coli*
Mikania micrantha	-	*8*	-	-	-	-	-
Cynodon *dactylon*	10	10	10	10	-	8	-
Borreria hispida	-	8	10	-	-	-	-
Oxalis corniculata	10	8	-	-	-	-	-
Oxalis acetosella	12	8	-	-	10	-	10
Chloramphenicol(C) 30mcg	15	-	-	30	-	8	-
Clotrimazole (CC) 10mcg	20	10	14	20	8	-	26
Ampicillin(AP) 10mcg	-	-	-	-	12	10	10

*- No activity. Zone of inhibition includes the diameter of well (6mm).

Table 5: Antifungal activity of the plants

Samples	*P. crysogenum*	*C. albicans*
Mikania micrantha	-	10
Cynodon dactylon	-	8
Borreria hispida	-	14
Oxalis corniculata	-	-
Oxalis acetosella	-	-
Nystatin (NS) 50mcg	-	24
Clotrimazole(CC) 10mcg	11	32
Ampicillin(AP) 10mcg	-	46

*- No activity. Zone of inhibition includes the diameter of well (6mm).

REFERENCES

1. Kubde MS, Khadabadi SS, Farooqui IA, Deore SL, Report and Opinion, 2(12), 2010, 24-31.
2. Bhoomika R Goyal, Ramesh K Goyal, Anita A Mehta, Pharmacognosy Reviews, 1(1), 2007, 143-150.
3. Santos PRV, Oliveria ACX, Tomassini TCB, Controle microbiogico de products fitoterapicos, Rev. Farm. Bioquim, 31, 1995, 35-38.
4. Krishnaraju AV, Rao TVN, Sundararaju D, Assessment of bioactivity of Indian medicinal plants using Brine shrimp (Artemia salina) lethality assay, Int J Appl Sci Eng., 2, 2005, 125-134.
5. Duraipandiyan V, Ayyanar M, Ignacimuthu S, Antimicrobial activity of some ethnomedical plants used by Paliyar Tribe from Tamil Nadu, India, BMC complementary and alternative medicine, 2006, 635.
6. Edeoga HO, Okwu OE, Mbaebie BO, Phytochemical constituent of some Nigerian medicinal plants, African journal of Biotechnology, 4(7), 2005, 685-688.
7. Aja PM, Okaka ANC, Onu PN, Ibiam U ,Urako AJ, Phytochemical composition of Talinum triangulare (water leaf) leaves, Pakistan Journal of Nutrition, 9(6), 2010, 527-530.
8. Ajayi IA, Ajibade O, Oderinde RA, Preliminary phytochemical analysis of some plant seeds, Research Journal of Chemical Science, 1(3), 2011.
9. Malik EP, Singh MP, Plant enzymology and hittoesnsymology, Kalyani Publishers, New Delhi, 1980, 286.
10. Mervat MMEIF, Hanan AA, Antioxidant activities total anthrocyanine, phenolics and flavonoids content of some sweet potato genotypes under stress of different concentrations of sucrose and sorbitol, Australian J. Basic Applied Sc., 3, 2009, 3609-3616.
11. Anti-Stanojevic L, Stanojevic M, Nikolic V, Nikolic L, Ristic J, Canadanovic, Brunet V, Antioxidant activity and total phenolic and Flavonoid contents of *Hieracium Pilosella* L. extracts, Sensors, 9, 2009, 5702-5714.
12. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C: Antioxidant activity: applying an improved ABTS radical cation decolorization assay**,** Free Rad. Biol. Med., 26, 1999, 1231-1237.
13. Nair R, Kalariya T, Chanda S, Antibacterial activity of some selected Indian medicinal flora, Turk. J. Biol., 29, 2005, 41-47.
14. Kaleewaran B, Ilavenil S, Ravikumar S, Screening of phytochemical properties and antibacterial activity of *Cynodon dactylon* L., International Journal of Current Research, 3, 2010, 83-88.
15. Rahee SA, Mallik J, Phytochemical screenings of the methanol extract of whole plant *Borreria articulari*. International Journal of Pharmaceutical & Biological Archives, 3(5), 2012, 1062-1066.
16. Raghavendra MP, Satish S, Raveesha A, Phytochemical analysis and antibacterial activity of *Oxalis corniculata;* a known medicinal plant, My Sci., 1, 2006, 72-78.
17. Shajiselvin CD, Muthu AK, *In-vitro* antioxidant studies of various extracts of whole plant of *Borreria hispida* (Linn), Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2010, 14-20.
18. Reddy KY, Saravana Kumar A, Lakshmi M, Angothu S, Antioxidant properties of methanolic extract of *Oxalis corniculata*. International Journal of Phytopharmacology, 1(1), 2010, 43-46.
19. Jananie RK, Priya V, Vijayalakshmi K, The quantitative analysis of the extract showed that the extract possessed a significant amount of total phenolic s than ascorbic acid and total flavonoids, Total antioxidant capacity, *In-vitro* assessment of free radical scavenging activity of *Cynodon dactylon,* 2011.
20. Hui Z, Rui-Jun MA, Shuang-Tao WU, Ultrasonic-assisted Extraction and Radical Scavenging Activity of Total Flavanoids from Different Parts of *Mikania micrantha,* J. Food Science, 31(6), 2010, 70-73.
21. Muthu AK, Sravanthi P, Kumar DS, Smith AA, Manavalan R, Evaluation of antibacterial activity of various extracts of whole plant of *Borraria hispida* (Linn), International journal of Pharm Sciences and Research, 1(2), 2010, 127-130.
22. Chethan J, Sampath kumara KK, Sekhar S, Prakash HS, Antioxidant, antibacterial and DNA protecting activity of selected medicinally important Asteraceae plants, International Journal of Pharmacy and Pharmaceutical Science, 4(2), 2012, 257-261.

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