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Research Article

Phytochemical Screening, Total Flavonoid Content and Antimicrobial Study of *M.spicata (Dalz.)Nicolson.*

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ABSTRACT

Medicinal plants have been playing a vital role on the health and healing of man since down human civilization. Inspite of tremendous development in the field of allopathic medicines during the 20th century, plants still remain one of the major sources of drugs in modern as well as in traditional system of medicines. Moullava spicata having potent antioxidant activity and many more medicinal properties. In present investigation phytochemical analysis, total flavonoid content and antimicrobial studies were carried out. It shows presence of phenolic compounds, saponins, terpenoids, flavonoids, glycosides and alkaloids. Ethylacetate extract shows antibacterial as well as antifungal activity. Flavonoid content in hydroalcoholic extract of m.spicata is also reported in present investigation.

Keywords: Phytochemicals; *m.spicata*; total phenolic content. antibacterial and antifungal activity.

INTRODUCTION

The importance of plants is known to us well. The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants [1]. Numerous studies have shown that aromatic and medicinal plants are sources of diverse nutrient and non nutrient molecules, many of which display antioxidant and antimicrobial properties and can protect the human body against both cellular oxidation reactions and pathogens [2]. Recently much attention has been focused on reactive oxygen species and free radicals which play an important role in the genesis of various diseases such as inflammation, liver cirrhosis, ischemia, cancer etc [3]. Flavonoids and phenolics are the bioactive phytoconstituents having an important role in control and prevention of tissue damage by activated oxygen species, hence herbal drugs containing such phytoconstituents are gaining importance in the prevention and treatment of various organ toxicities due to environmental challenges [3].

Medicinal plants have been playing a vital role on the health and healing of man since down human civilization. Inspite of tremendous development in the field of allopathic medicines during the 20th century, plants still remain one of the major sources of drugs in modern as well as in traditional system of medicine. Plant derived medicines have made large contributions to human health. They may become the base for the development of medicine, a phytomedicine to be used for the treatment of diseases [4].Traditional medicine using plant extracts continues to provide health coverage for over 80% of the worlds population especially in the developing world (WHO,2002).

Recent attempt has been made to extract the moullava spicata plant material in various solvents. Moullava spicata is called as candy corn plant, it is large robust climber belonging to family caesalpinaceae [5,6].The important feature of this plant is that it is indigenous to western presidency [8]. Pods of this plant contain large proportion of Tannic acid[7,8,9].Bark is having application for skin diseases[7,8,9]. Ethanolic extract of aerial parts exhibits hypotensive activity [9].Seeds of this plant contains oil, which is used as an illuminant and this plant also used to cure diarrhea [9].The extracts of this plant shows superoxide and hydroxyl radical scavenging activity[10,11].It has been reported that this plant extracts having presence of saponins, phenolic compounds, flavonoids, glycosides, steroids, terpenoids and also quantification of total phenolic component is reported [12]. Extracts of this plant having potent antioxidant property[12].

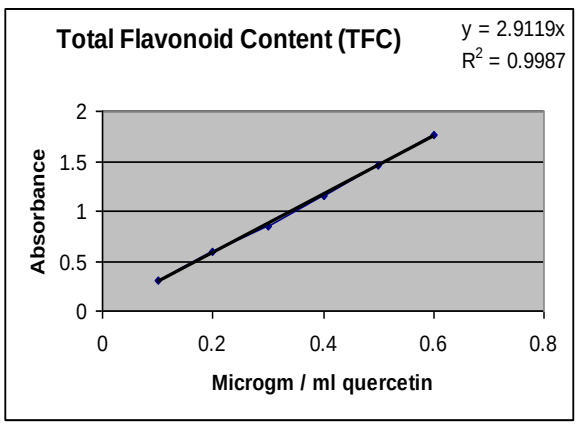

Fig. 1 Calibration plot for flavonoid determination.

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Table 1: Antibacterial activity (By Paper diffusion Method).

Bacterial stain used	Methanol Extract	Ethyl acetae Extract	Pet.ether Extract
Staphylococcus aureus	--	1.7 cm	--
Control	--	--	--
Salmonella typhosa	--	1.7 cm	2.1 cm
Control	--	1.4 cm	1.4 cm
Pseudomonas aeruginosi	--	--	--
Control	--	--	--
E.coli	--	--	--
Control	--	--	--

Table 2: Antibacterial activity (By Broth dilution method).

ANTIBACTERIAL ACTIVITY TABLE
MINIMAL INHIBITION CONCENTRATION

SR NO.	STAIN	E. COLI	P. AERUGINOSA	S. AUREUS	S.PYOGENUS
	CODE NO.	MTCC 442	MTCC 441	MTCC 96	MTCC 443
			[MICROGRAM/ML]		
1	MSMEE	12.5	200	250	200
2	MSEAE	62.5	100	125	100
3	MSPEE	200	125	100	125

MSMEE: Methanol Extract.
MSEAE : Ethyl acetate Extract.
MSPEE: Petroleum ether Extract.

A thorough survey of literature revealed no antibacterial reports on moullava spicata. Total flavonoid content, phytochemical analysis and antimicrobial study is carried out. Despite of a long tradition of use for the treatment of various ailments, no systematic phytochemical and pharmacological work has even been carried on this potentially useful plant, thus the present investigations were planned with an objective to estimate total flavonoid content in hydroalcoholic extract of m. spicata, phytochemical screening of ethylacetate, methanol and hydroalcoholic extracts and antimicrobial evaluation of petroleum ether, ethylacetae and methanol extracts of m.spicata.In present investigation antibacterial study was carried out by two methods i.e. by paper diffusion method and broth dilution method.Fresh plant material was collected from Kolhapur and authenticated at ARI, Pune . Quercetin (Hi media Lab. Pvt Ltd, Mumbai) was used as standard drug for determination of flavonoid content.All solvents used in present investigation were of AR Grade, procured from S.D.fine chemicals, Mumbai.

MATERIALS AND METHODS

Collection and Identification of plant material: Fresh plant material of m.spicata was collected from Dajipur, Radhanagari, Dist. Kolhapur (MS) India in month of Nov.2011 and was identified by Dr. Mrs. A.S. Upadhye, Scientist B, Plant Science Division, Agharkar Research Institute, Pune (MS) India.

Extraction of plant material: The fresh plant material was air dried at room temperature for two weeks, after which it was grinded to a uniform powder and stored in an air tight container for further use. Exactly 400gm powdered plant material was extracted in soxhlet apparatus with 2 lit of petroleum ether (60 – 80°C) for 24 hours. The extracted plant material (marc) was air dried and again extracted in same soxhlet apparatus with ethylacetae, methanol & then 80% ethyl alcohol. The extracts were filtered through whatmann filter paper no.42 (125mm) and then through cotton wool. The extracts were concentrated using a rotary evaporator with water bath set at 40°C. The solid extracts were stored at cold condition for further use.

Phytochemical Screening: Phytochemical screenings were performed using standard procedures given in J. of Phytology [1]. Ethylacetae, methanol and 80% ethylalcohol extract (hydroalcoholic extract) were tested for carbohydrates, phenols and tannins, flavonoids, saponins glycosides, steroids, terpenoids and alkalods.

Determination of total flavonoids: The aluminium chloride method was used for the determination of total flavonoid content[13,14].Aliquot of the extract solution (0.1ml) was taken in glass tube and made upto the volume 2 ml with methanol, then 0.5 ml (1.2%) $AlCl_3$, 0.5 ml (120mM) potassium acetate were added. The test solution was vigorously shaken; absorbance at 415 nm was measured after 30 minutes of incubation. A standard calibration plot was generated at 415 nm using known concentrations of quercetin.The concentration of flavonoid in the test sample was calculated from the calibration plot and expressed as mg quercetin equivalent / gm of sample.

Antibacterial and antifungal activity: Test microorganisms used to study antibacterial activity by paper diffusion method are staphylococcus aureus, salmonella typhosa, pseudomonas aeuruginosii and E.coli. The bacterial stains used for this study are available at

DRUG	E. COLI	P. AERUGINOSA	S. AUREUS	S.PYOGENUS
	MTCC 442	MTCC 441	MTCC 96	MTCC 443
	[MICROGRAM/ML]			
GENTAMYCIN	0.05	1	0.25	0.5
AMPICILIN	100	--	250	100
CHLOROAMPHENICOL	50	50	50	50
CIPROFLOXACIN	25	25	50	50
NORFLOXACIN	10	10	10	10

Table 3: Antifungal activity (By Broth dilution method).

ANTIFUNGAL ACTIVITY TABLE MINIMAL FUNGICIDAL CONCENTRATION				
SR.NO	CODE NO	C.ALBICANS	A.NIGER	A.CLAVATUS
		MTCC227	MTCC282	MTCC1323
	[MICROGRAM/ML]			
1	MSMEE	1000	500	1000
2	MSEAE	250	500	1000
3	MSPEE	500	1000	> 1000

DRUG	C.ALBICANS MTCC227	A.NIGER MTCC282	A.CLAVATUS MTCC1323
	[MICROGRAM/ML]		
NYSTATIN	100	100	100
GRESEOFULVIN	500	100	100

Microbiology department, T.C.College, Baramati, Dist.Pune (MS) India. Experiments carried out in same laboratory.Paper diffusion method were used for study and DMSO is used as control.

Antibacterial and antifungal activity was carried out by Broth dilution method. Bacterial stains used are E.Coli (MTCC 442), p.auriginosa (MTCC 441), s.aureus (MTCC 96), s.pyogenus (MTCC 443) and standard drugs used are Gentamycin, Ampicillin, Chloamphenicol, Cipprofloxin and Norfloxin. Antifungal stains used are C.albicans (MTCC 227), A.niger (MTCC 282), A.clavthus(MTCC 323), and standard drugs used are Nystatin and Greseofulvin. All these stains and standard drugs are available at Microcare Laboratory, Surat, Gujrath, India and these experiments were carried out in same laboratory. The standard protocols are used during the antimicrobial study [15, 16, 17].

RESULTS AND DISCUSSION

Phytochemical screening: Phytochemical screening of ethylacetae, methanol and hydroalcoholic extracts of m.spicata shows presence of saponins, glycosides, terpenoids, alkaloids, carbohydrates, flavonoids, phenols and tannins etc.

Total flavonoid content: The present study reveals the flavonoid content of the extract of m.spicata in terms of mg quercetin equivalent / gm of dry sample. Flavonoids are one of the most diverse and wide spread group of natural compounds are probably the most important phenols[13].These compounds possesses a broad spectrum of chemical and biological activities including radical scavenging properties [12].Using standard plot of quercetin ($y = 2.991x$, $R^2 = 0.9987$) the flavonoid content of this extract is found to be 0.529 mg quercetin equivalent / gm dry sample.

Antibacterial activity

All three extracts of this plant showed varying degree of antibacterial activities against the test bacterial species. In paper diffusion method DMSO is used as control. Ethylacetate extract shows 1.7 cm inhibition zone for staphylococcus aureus, 0.3 cm for salmonella typhosa, petroleum ether shows 0.7 cm inhibition zone for salmonella typhosa. It reveals that ethylacetate extract having some antimicrobial components.

Control used: DMSO.

By Broth dilution method ethylacetate extract shows comparable results with E.coli , S.aureus and S.pyogenus.

Antifungal activity: Out of three extracts ethyl acetate and pet. Ether extracts shows comparable results with C.albicans.

CONCLUSION

In present study the total flavonoid content, antimicrobial activity and phytochemical analysis were done and this is helpful in gauging the potential bioactivities of this plant. In addition to this present findings are not only helpful for establishing the phytochemical standardization but also in authentication of the drugs, more experiments are needed to isolate bioactive phytoconstituents from this plant.

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REFERENCES

1. Yadav, RNS. Agarwala, M., Phytochemical analysis of some medicinal plants, Journal of phytology, 2011; 3(12), 10-14.
2. Patil , N. B., Adsul, V.B., Khatiwaora,E., Kale,A.A., Tambe,S.P., Deshpande,N.R., Spectroscopic Determination of total phenolic and flavonoid contents of Tribulus terrestris fruits, International Journal of Chem Tech Research, July – Sept. 2012,4(3),899-902.
3. Ntraj,H.N., Murthy,R.L.N.,Setty,R.S.,Invitro quantification of flavonoids and phenolic content of Suran, International Journal of Chem Tech Research, Oct-Dec 2009, 1(4), 1063 - 1067.
4. Igbinosa O.O., Igbinosa E.O., Aiyegoro O.A., Antimicrobial activity and phytochemical screening of stem bark extracts from Jatropa Curcas (Linn), African Journal of Pharmacy and Pharmacology, Feb 2009,3(2), 058-062.
5. Cooks, The flora of Bombay Presidency, Taylor and Francis , London 1903, 1,416.
6. Kirtikar, K.R., Basu, B.D., Indian medicinal plants, Lalit Mohan Basu, Allahabad, 1933 ,2, 853.
7. Chopra, R.N., Nayar, S.L., Chopra, I.C., Glossary of Indian Medicinal Plants, Council of Scientific and Industrial research, New Delhi,1956,257.
8. Nadkarni, K.M., Indian Materia Medica, Vol.I, Bombay Popular Prakashan, 1976, 1290.
9. The useful plants of India, Anonamous, Council of Scientific and Industrial Research, New Delhi, 1992,686.
10. Joshi, D.V., Tamhane, R.G., Datta,N.K., Current Science, 1957, 26(25), 147-148.
11. Shamak,G., Shenoy, R.P., Manjunatha, S.M., Vinayak, K.S., Food Chemistry, 2009, 115 (2), 631-634.
12. Lohith,K., Vijay,R., Pushpalatha,K.C., Joshi,C.G., Phytochemical and Antioxidant evaluation of moullava spicata (Dalzell) Nicolson leaf extract, Annual Research and Review in Biology,2014, 4(1),188-197.
13. Eghdami,A., Sadeghi,F., Determination of total phenolic and flavonoid contents in methanolic and aqueous extracts of Achillea Millefolium, Organic Chemistry Journal, 2010, 2, 81-84.
14. Lobo Richard., Sodde Vijay., Dashora Nipun., Gupta Nilesh., Prabhu Kirti., Quantification of flavonoid and phenol content from macrosolen parasiticus (L.) Danser, J.Nat.Prod. Plant Resour; 2011,1(4) ,96-99.
15. Isenberg,H.D., Clinical Microbiology Procedure Handbook, 2nd Edition, Chapt.5, page 5.0.1.
16. Indian Journal of Chemistry, section – b, vol 46 b, March 2007, 550-553.
17. Wayne, P.A., National Committee for Clinical Laboratory Standards,5th edition,2000.
18. Shadomy, S., Mannual of Clinical Microbiology., Albert, B., Ed: ASM press; Washington, DC, 1991, 1173.
19. Rattan, A., Antimicrobials in Laboratory Medicine., BI Churchill Livingstone., India,2000,85.
20. Sadasivam,S., Manikam, A., Biochemical Methods,3rd edition,New Age International Publishers, New Delhi,2008,203-211.
21. Lohith, K., Vijay, R., Joshi K.C., Chandrasekhar G., In vitro cytotoxic study of moullava spicata (Dalz.) Nicolson leaf extract, Indian journal of forensic medicine and toxicology, July-Dec 2013, 7(2), 182.