ISSN : 2320 4850

BI MONTHLY

Asian Journal of Pharmaceutical Research And Development

(An International Peer Reviewed Journal of Pharmaceutical Research and Development)

AJPRD

Volume - 01 Issue - 03 MAY-JUN 2013

website: www.ajprd.com

editor@ajprd.com

Asian Journal of Pharmaceutical Research and Development
(An International Peer-Reviewed Journal of Pharmaceutical Research and Development)
www.ajprd.com
ISSN 2320-4850

Research Article

PRELIMINARY PHYTOCHEMICAL AND PHENOLIC CONTENT OF STEMS BARK OF *ABELMOSCHUS MANIHOT* (LINN.) MEDIK

Chumbhale D. S.[1]*, Chaudhari S. R.[1], Upasani C. D.[2]

[1]Department of Pharmacognosy, Amrutvahini College of Pharmacy, Sangamner-, Maharashtra, India.
[2]SNJB'S Shriman Sureshdada Jain College of Pharmacy, Chandwad, Nashik – , Maharashtra, India.

Received: 01May 2013, ***Revised and Accepted: 10 May 2013***

ABSTRACT

The present study was designed to determine the phytochemical properties and phenolic content of various extracts of the Abelmoschus manihot (Linn.) Medik (A. manihot) is an important medicinal plant in Indian traditional system of medicine. Phenolic content of A. manihot stems bark extracts was determined by Folin-coutigue reagent test. The extractive values of petroleum ether, methanolic, ethyl acetate soluble fraction of methanolic extract, ethyl acetate insoluble fraction of methanolic extract and aqueous extract were found to be 3.75%, 8.00%, 3.80%, 4.20%, 8.44% w/w respectively. Preliminary phytochemical analysis mainly revealed the presence of carbohydrates, glycosides, flavonoids, tannins, steroids and proteins in methanolic, ethyl acetate soluble fraction of methanolic extract, ethyl acetate insoluble fraction of methanolic extract and aqueous extracts. Petroleum ether extract shows positive result for the fixed oil and fats, saponin and phytosterol. Phenolic content of petroleum ether, methanolic, ethyl acetate soluble fraction of methanolic extract, ethyl acetate insoluble fraction of methanolic extract and aqueous extracts were found to be 16.00%, 24.00%, 28.00%, 28.00%, 38.00%, 32.00% respectively. Present study of preliminary phytochemical screening and phenolic content of A. manihot stem bark provide useful information which may help in authenticating the genuine plant along with nature of phytoconstituents present in it. These findings will be useful towards further isolation of phytoconstituents from various extract of A. manihot stems bark and support for the safe use of this traditional plant.

Key words: *Abelmoschus manihot, Malvaceae, Traditional medicine, Phytochemical, Phenolic content*

INTRODUCTION

A large annual erect hairy herb or undershrub, represented by *Abelmoschus manihot* (*A. manihot*) (L.) Medik (Malvaceae) Synonym: *Hibiscus manihot* Linn is medicinally important plant of the Malvaceae family commonly known as '*Jangali bhendi*' is a 1.2-1.8 m. high, commonly found on the Kokan, Western Ghats and Western coasts of India [1].

*For Correspondence:
Deshraj Shyamkant Chumbhale*
Assistant Professor, Department of Pharmacognosy
Amrutvahini College of Pharmacy,
Sangamner, Ahmednagar -422 608, Maharashtra, India.
Mail id: deshraj_sk@rediffmail.com.

It is an annual, erect hairy herb or undershrub having stems with small scattered prickles. Leaves 9 cm. long, scabrid with short stiff hairs, cordate, serrate, acutely angled or more or less palmately 5-7 lobed, lobes again variously divided, usually acuminate, petioles 5-12.5 cm. long, prickly. Stipules 1 cm. long, linear lanceolate, with stiff bristles on the margins. Pedicels less than 2.5 cm. long axillary and clustered at the ends of the branches, stout, sometimes with a few prickles. Calyx softly villous, within and without, ovoid, acuminate in bud, 2.2cm. long, Carolla 5-7.5 cm. across, yellow with purple centre. Capsule 3.8 cm. long, ovoid, 5- angled, hispid, cuspidate. Seeds faintly pubescent. Roots of this plant having yellowish brown

color with 3-6 cm long, wavy shape [2]. In the folk medicine, this plant has been known since ancient times for its curative properties and has been utilized for treatments of various ailments such as bark is considered as an emmenagogue and used to treat wounds and cuts. Traditionally the bark of this plant is used as an anthelmintic, febrifuge, alexeteric and diuretic. Bark is also used for treating strangury and urinary complaints [2- 3]. Root paste and leaves useful for boils, sores, sprains, inflammations, tuberculosis and leucoderma [3-6]. The juice of the flowers is used to treat chronic bronchitis and toothache [7]. The leaves of this plant reported for antinflammatory activity [8, 9]. Leaves showed bone-sparing effect [10]. Flowers reported as a neuroprotective and antiviral [11-12]. Stems reported for wound healing activity [13]. Roots of this plant reported for larvicidal and analgesic activity [14, 15]. The aerial part reported for dampness-heat of glomerulonephritis, diabetic nephropathy and presence of mosquito larvicidal compound [16-18]. A review has mentioned the phytochemical properties of different parts constituted a wide range of chemical compounds such as stems of *A. manihot* reported for presence of stigmasterol and γ-sitosterol [19]. Flowers are reported for hyperoside and flavanoids includes hibifolin, quercetin and myricetin derivatives [20]. The literature survey and screening of scientific data revealed that although *A. manihot* stem bark are traditionally used in the treatment of various diseases for long time, no systematic phytochemical studies are reported for the stem bark of this plant. Therefore present investigation was planned to study the preliminary phytochemical evaluation and phenolic content of various extracts of stem bark of *A. manihot*.

MATERIALS AND METHODS

Plant material

The plant, *A. manihot* was collected in Trimbakeshwar Hills, Nashik District, Maharashtra in May 2011. The plant was authenticated by Dr. Mrs. A. S. Petkar at the Department of Botany and herbarium deposited in S. N. Arts, D. J. M. Commerce & B. N. S. Science College, Sangamner, Ahmednagar, Maharashtra, India under voucher specimen number CDSAM3 (No.BNS/Tech/2011/164). The stem bark of the plant were dried, powdered and passed through 40 mesh sieve and stored in an airtight container for further use.

Preparation of extract

The air-dried stem barks of the plant, *A. manihot* was made into a coarse powder. The powdered material was defatted with petroleum ether. The defatted material was successively extracted with methanol and distilled water using a Soxhlet extractor. Methanolic extract was further fractionated with ethyl acetate to get ethyl acetate soluble and ethyl acetate insoluble fractions. Then the extract was filtered through muslin and the filtrate was evaporated under reduced pressure and vacuum-dried [21].

Preliminary phytochemical studies

Preliminary phytochemical studies of various extracts of *A. manihot* stem bark was carried out by performing qualitative chemical test as per standard procedure [22].

Estimation of phenolic content

100 mg of extract was added into 40 ml ethanol and then was mixed and sonicated for about 30 min and shaked about 10 min. The volume was made up to 100 ml with HPLC grade water. Mixed well, this solution was filtered with No.1 Whatman paper. An aliquot of this solution was mixed with 0.5 ml of Folin-Ciocalteu Phenol reagent. After 5 min, 1.5 ml of 20% sodium carbonate solution was added and the volume was made up to 10 ml with HPLC grade water. After 2 h, the solution was filtered with No.1 Whatman paper and the absorbance at 760nm was recorded. The same solution without the extract solution was used as blank solution. The blank was similarly prepared without using any extract. The standard solutions were prepared and analyzed by the same manner using 20mg of accurately weighted Gallic acid. The same solution without Gallic acid was used as the blank solution. Calculation of content of total

phenols in percent was based on Gallic acid standard. Total Phenols % = Absorbance (sample) X Weight (standard) X 100 /Absorbance (standard) X Weight (sample) [23].

RESULTS

In present study the extractive values of petroleum ether, methanolic, ethyl acetate soluble, ethyl acetate insoluble and aqueous extract were found to be 3.75%, 8.00%, 3.80%, 4.20%, 8.44% w/w respectively. The extractive values are shown in Table 1**.** Preliminary Phytochemical screening including qualitative chemical examination of various extracts of *A. manihot* stem bark reveals the presence of carbohydrates, glycosides, tannins, gums and mucilage, saponin, phytosterol, flavonoids and phenolic compounds in methanolic extract, ethyl acetate soluble fraction of methanolic extract, ethyl acetate insoluble fraction of methanolic extract and aqueous extracts. Petroleum ether extract shows positive result for the fixed oils and fats, saponin, and phytosterol. The results are shown in Table 2.

Phenolic content of petroleum ether, methanolic, ethyl acetate soluble fraction of methanolic extract, ethyl acetate insoluble fraction of methanolic extract and aqueous extracts were found to be 16.00%, 24.00%, 28.00%, 28.00%, 38.00%, 32.00% respectively. The results are shown in Table 3. The calibration curve of total phenolic content of standard gallic acid (Concentration Vs Absorbance) is shown in Figure 1.

Table 1: Percentage yield of extracts of A. manihot stem bark

Extract	Yield (% w/w)	Color of extract
Petroleum Ether	3.75	***Yellow***
Methanolic	8.00	***Reddish brown***
Ethyl acetate soluble	3.80	***Reddish brown***
Ethyl acetate insoluble	4.20	***Brown***
Aqueous	8.44	***Brownish black***

DISCUSSION

Phenolic compounds are a large, heterogeneous group of secondary plant metabolites that are widespread in the plant kingdom [24]. Polyphenols are the products of plant metabolism and can range from simple molecules to highly polymerized compounds. Phenolics display a vast variety of structures; here only flavonoids, tannins and phenolic acids are reviewed. Flavonoids, a subclass of polyphenols, are the most common polyphenolic compounds found in nature and are further divided into several subclasses including flavones, flavonols, isoflavones, anthocyanins, flavanols, and proanthocyanidins. Flavonoids and other plant phenolics are especially common in leaves, flowering tissues and woody parts such as the stem and bark [25]. In present study of preliminary phytochemical screening and phenolic content analysis of various extracts showed positive test for phenolic constituents and its important abundance in stem bark of *A. manihot*.

Table 2: Phytochemical screening of different extracts of A. manihot stem bark

Chemical Constituents	*Chemical tests*	*Petroleum ether extract*	*Methanol extract*	*Ethyl acetate soluble fraction*	*Ethyl actate Insoluble fraction*	*Aqueous extract*
Alkaloids	Dragendorff's test	–	–	–	–	–
	Mayer's reagent	–	–	–	–	–
Carbohydrates	Molisch's test	–	+	–	+	+
	Barfoed's test	–	+	–	+	+
Glycosides	Borntrager's test	–	+	+	+	+
	Keller-killianin test	–	+	–	–	–
Saponin glycosides	Foam test	+	–	–	–	–
Flavonoids	Shinoda Test	–	+	+	+	+
	Sodium hydroxide test	–	+	+	+	+
Fixed oils & fats	Sudan red III	+	–	–	–	–
Tannins& Phenolic compounds	Ferric chloride test	–	+	+	+	+
	Phenazone test	–	+	+	+	+
Steroids	Salkowaski test	+	+	–	–	–
	Libermann-burchard test	+	+	–	–	–
Proteins	Biuret test	–	–	–	–	+

Table 3: Total phenolic content of various extracts of A. manihot stem bark

Sr. No.	*Extract*	*Total Phenolic Content*
1	Petroleum ether	16.00%
2	Ethyl acetate soluble	24.00%
3	Ethyl acetate insoluble	28.00%
4	Aqueous	32.00%
5	Methanol	38.00%

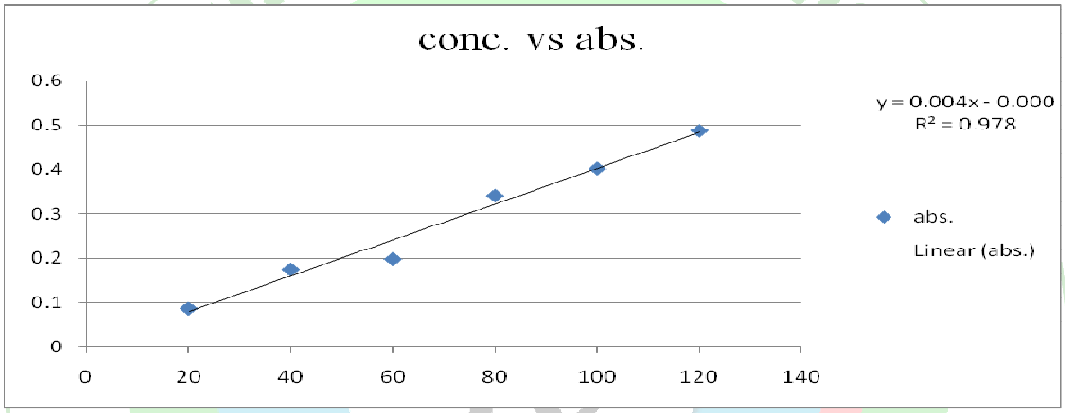

Fig.1: Calibration curve of total phenolic content of Gallic acid

CONCLUSION

Present investigation has to indicate the scientific evidences and useful information which may help in authenticating the genuine plant along with nature of phytoconstituents present in it. These findings will be useful towards further isolation of phytoconstituents from various extract of *A. manihot* stems bark and support for the safe use of this traditional plant.

ACKNOWLEDMENT

I express my sincere thanks to Management, Amrutvahini College of Pharmacy, Sangamner, who took interest in looking into our research needs and thus providing us with the best available resources.

REFERENCES

1. *Anonymous, The Wealth of India a dictionary of Indian raw material and industrial products, Vol V: R-Z. New Delhi: Council of Scientific and Industrial Research, 2005; p. 90-91.*
2. *Kirtikar KR, Basu BD. Indian Medicinal Plants, Vol I. 2nd ed. Dehradun: International book Distributors, 2005; 339.*
3. *Manandhar NP. Ethnobotanical note on folklore remedies of Baglung District, Nepal, Contribution to Nepalese Studies 1993; 20:2: 183-96.*
4. *Chopra RN. Glossary of Indian Medicinal Plants, Part I A-K. New Delhi: Council of Scientific and Industrial Research, 2001; 1-2.*
5. *Sharma P, Mishra N. Ethno-medicinal uses and agro-biodiversity of Barmana region in Bilaspur district of Himachal Pradesh, Northwestern Himalaya, Ethnobotanical Leaflets, 2009; 13: 709-21.*
6. *Patil HM, Bhaskar VV. Medicinal knowledge system of tribals of Nandurbar district, Maharashtra, Indian Journal of Traditional Knowledge, 2006; 5:3: 327-30.*

7. *Manandhar NP. Plants and people of Nepal, Oregon: Timber Press; 2002.p.877-91.*
8. *Jain PS, Bari SB. Anti-inflammatory activity of Abelmoschus manihot extracts, International Journal of Pharmacology, 2010; 6:4: 501-05.*
9. *Winter CA, Risley EA, Nuss WG. Carrageenin-induced edema in hind paws of the rats as an assay for anti-inflammatory drugs, Proceeding of Society of Experimental and Biological Medicine, 1962; 111: 544-47.*
10. *Puel C, Mathey J, Davicco MJ, Lebecque P, Chanteranne B, Horcajada MN, Coxam V. Preventive effect of Abelmoschus manihot (L) Medik on bone loss in the overiectomised rats, Journal of Ethnopharmacology, 2005; 99: 655-60.*
11. *Cheng XP, Qin S, Dong LY, Zhou JN. Inhibitory effect of total flavone of Abelmoschus manihot L. Medic on NMDA receptor-mediated current in cultured rat hippocampal neurons, Neuroscience Research, 2006; 55:2: 142-45.*
12. *Lin-lin WU, Yang X, Huang Z, Liu H, Guang WU. In vivo and in vitro antiviral activity of hyperoside extracted from Abelmoschus manihot L. Medic, Acta Pharmacological Sinica, 2007; 28:3: 404-9.*
13. *Jain PS, Bari SB. Evaluation of wound healing effect of Abelmoschus manihot in rats, Revista Brasileira de Farmacognosia, 2010; 20:5: 756-61.*
14. *Virendra KD, Pandey AC, Alam MF, Dash AP. Larvicidal activity of Hibiscus abelmoschus against mosquitoes. Journal of American Mosquito Country Association, 2006; 22:1: 155-57.*
15. *Goyal N, Sharma SK, Singh S, Vasudeva N. Analgesic activity of root of Abutilon indicum Linn, Hamdard Medicus, 2006; 49:4: 14-16.*
16. *Yu JY, Xiong NN. Pathogenic factor (Dampness-heat) of glomerulopathy, Zhongguo Zhong Xi Yi Jie Za Zhi, 1992; 12: 458-60.*
17. *Yu JY, Xiong NN, Guo HF. Clinical observation on diabetic nephropathy treated with alcohol of Abelmoschus manihot, Zhongguo Zhong Xi Yi Jie Za Zhi, 1995; 15:5: 263-265.*
18. *Abdul Rahuman A, Gopalakrishnan G, Venkatesan P, Geetha K. Isolation and identification of mosquito larvicidal compound from Abutilon indicum (Linn.) Sweet, Parasitology Research, 2008; 102:5: 981-88.*
19. *Jain PS, Bari SB. Isolation of stigmasterol and γ – sitosterol from petroleum ether extract of woody stem of Abelmoschus manihot, Asian Journal of Biological Sciences, 2009; 2:4: 112-17.*
20. *Lai XY, Zhao YY, Liang H. Studies on chemical constituent in flower of Abelmoschus manihot. Journal of Integrative Plant Biology, 2009; 31:19: 1597-1600.*
21. *Mukherjee PK. Quality Control of Herbal Drugs – An Approach to Evaluation to Botanicals, 1st ed. New Delhi: Business Horizons Pharmaceutical Publishers, 2002; 379-425.*
22. *Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy, 39rd ed. Pune: Nirali Prakashan, 2007; 607-11.*
23. *Mc Donalds Prenzler PD, Autolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts, Food Chemistry, 2001; 73: 73-74.*
24. *Strube M., Dragstedt LO, Larsen JC. Naturally Occurring Antitumourigens. I. Plant Phenols, Copenhagen: The Nordic Council of Ministers, 1993; 39-40.*
25. *Larson RA. The antioxidants of higher plants, Phytochemistry, 1988; 27: 969-78.*