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Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Short Communication

PHYTOCHEMICAL SCREENING OF FRUIT AND STEM BARK EXTRACTS OF *LIMONIA ACIDISSIMA* (L.)

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ABSTRACT

Limonia acidissima Linn, syn. *Feronia limonia* is a member of family Rutaceae. Products made from stem bark and fruits of wood apple have been used in India for their medicinal properties. It contains pharmacologically bioactive compounds which are used for curing of various human diseases and also play an important role in diarrheoa and dysentery. Wood apple products are believed by Ayurvedic practitioners to be and antibacterial, antiviral, anti-diarrheal, antidiabetic activities. Primary phytochemical constituents include proteins, amino acids, sugar and carbohydrates. Secondary constituents contain terpenoids, saponins, flavonoids, steroids, glycosides, tannins and alkaloids. In the present study three different solvents were used *viz.* methanol, petroleum ether and chloroform. Qualitative phytochemical investigations for stem bark and fruits were carried out and results about occurrence were reported. During investigation, presence of flavonoids and glycosides were reported for all three solvents systems of fruits extract. This combination of the phytochemicals creates possibility of the justification of the claimed as well as prospective medicinal applications.

Keywords: *Limonia acidissima*, Phytochemicals, Extraction, Fruits.

1. INTRODUCTION

For the medication of diverse diseases, production of new drugs is important by medicinal plant. The phyto-chemical investigations of medicinal plants are valuable and it has profitable interest in both pharmaceutical companies and research institute. The fruit of *Limonia acidissima* has got extremely high medicinal benefits [1]. The preliminary phyto-chemical investigation tests may be necessary in the revelation of the bioactive compounds among them may be advantage for the drug finding and advancement [2]. *Limonia acidissima* L. belongs to family Rutaceae, and very rich source of vitamin C and flavonones, which belongs to flavonoid group [3]. It is most important medicinal plant which leads to the many applications for various disorders [4]. Wood apple contain hypoglycemic activity, hepato-protective activity, antitumor activity, antimicrobial activity and larvicidal activity [5]. It is multi stems tree, with placed in temperate regions and tropical regions of the whole world. The tree has rough spiny bark and leaves are pinnate. Stem bark is useful in treating liver disease [6] and gummy substance from the stem is useful in treating haemorroids [7]. Fruit of the tree is sweet either sour, it is a berry and 5 to 9 cm in width. The unripened fruits act as astringent and is useful for the diarrhea and dysentery. With the combination of beal and other medicines [8, 9]. Locally plant of *Limonia acidissima* known as kaith, kavit, kapitha and wood apple [10]. In the present study, extracts of three different solvents (methanol, petroleum ether and chloroform) from stem bark and fruits have been subjected to qualitative investigation of phytoconstituents. Analysis revealed the presence of flavonoids and glycosides were reported for all three solvents systems of fruits extracts.

2. MATERIAL AND METHODS

2.1. Collection of plant material

Fresh plant parts such as stem bark, and fruits were collected from Ratapani reserve, District Bhopal and were authenticated by Dr. G.P. Sinha (Scientist F & Head of office) Botanical Survey of India, Allahabad. The voucher specimen no (10433) was deposited at the Herbarium of Botanical Survey of India, Allahabad. Whole quantity of collected plant parts were cleaned

three times with the tap water and two times through the distilled water for taken out the adhering substances with different associated organisms.

2.2. Chemical and reagents

All the chemicals were used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (India) and SRL Pvt. Ltd. (India).

2.3. Preparation of plant extract

Plant parts were collected and dried at room temperature (20°C) for two to three weeks. Plant samples were cut up into the smaller pieces and then ground into the powder. Sample powder was stocked in the airtight polythene at room temperature up till necessary for extraction. Protocol of Nemlin and Brunel was used for this exercise [11]. Dried samples were exposed in 90% various organic solvents such as chloroform, petroleum ether and methanol in a soxhlet apparatus (Borosil). Soxhlet extraction was used for separation of components based on the differences in the solubility in the solvents. The powdered form of plant material (50gm) was fixed in the soxhlet extractor flask. On other hand 500 ml of the organic solvent was carried out in the round bottom flask. The soxhlet extraction was done continuously at convenient temperature for 6 to 8hrs. The extracts obtained were filtered and then each of the extracts was concentrated using rotary vacuum evaporator. The individual extracts were seized out in bottom flask which was heated at suitable temperature on a water bath. The vapors of the solvent rise in the condenser and after condensation the solvent droplets was collected in the collecting flask. The resultant sticky mass was collected in the crucible. In the oven, it was dried at low temperature. The obtained solid mass was stored in appropriate volume of 10% dimethyl sulphoxide (DMSO) along with in a drop of Tween-20 and filtered through milipore sterile filters (mesh 0.20 µm, sartorious stedim Biotech Gmbh, Germany). Filter paper packets of 50 gm of individual plant parts were prepared. These packets were place separately in 200 mL of hot water contained in bottles. The extraction was carried out for 24°C with intermittent shaking. The extracts obtained were concentrated and dried mass obtained was stored in a suitable volume of 10% dimethyl sulphate (DMSO) with a drop of tween-20. Phytochemical investigations were carried out for the presence of phytochemical constituents in the extracts by applying the standard method of Trease and Evans [12]. The percentage of extraction was calculated by adopting the following formula [13].

Yield (%) = Dry weight of extract/Dry weight of plant powder×100

2.4. Phytochemical analysis

Phytochemical screening of the stored plant extracts were carried out for the presence and absence of the primary and secondary metabolites. For screening of phytoconstituents, standard procedures were employed [14]. The extract of the plant parts were tested for the presence and absence of active constituents such as alkaloids, saponins, glycosides, steroids, terpenoids, tannins and flavonoids, phenolic compounds and carbohydrates etc. The phytoconstituents result were indicated as (+) presence and (-) absence

3. RESULTS AND DISCUSSION

Different solvents, based on their polarity were used for the extraction of two parts stem bark and fruits of *Limonia acidissima* for phytochemical analysis. The result of the extraction revealed the presence of various phytochemical constituents. Qualitative phytochemical screening of chloroform extract, petroleum ether extract and methanol extract of stem bark and fruits showed in table 1**.** Phytochemical constituents such as alkaloids, flavonoids, tannins, steroids, terpenoids, glycosides, saponins, carbohydrates and phenolic compounds were found to present and absent in the plant extract. Methanol extract of stem bark and fruit showed the presence of alkaloids, flavonoids, steroids, saponins glycosides, carbohydrates, phenolic compounds and absence of tannins besides terpenoids only present in methanolic extract of fruits and absent in stem bark. Petroleum ether extract of stem bark and fruits showed the presence of tannins, flavonoids, terpenoids and absence of alkaloids, steroids, saponins, phenolic compounds and carbohydrates while glycosides only present in fruits not in stem bark of petroleum ether extract. Chloroform extract of stem bark and fruits showed the presence of alkaloids, glycosides and absence of tannins and terpenoids in both. While chloroform extract of stem bark shows the presence of steroids, phenolic compounds, carbohydrates and only fruits showed the presence of saponins, flavonoids. Preliminary phytochemical investigation were showed that Alkaloids, Tannins, Flavonoids, Terpenoids, Steroids, Saponins,

Glycosides, carbohydrates and phenolic compounds were present and absent in the stem bark and fruits extracts. The occupancy of these secondary metabolites in the plants generates few biological activity in human and animals. It is also responsible for their uses as herb in primary health care system [15]. These compounds also supply as to protect the plant against infections by microorganisms predations by insects and herbivores, while their flavor and odor are responsible for their pigments [16]. Antibiotic resistance is a large-scale concern along with development of new drug agents from plant it could be beneficial in making the demand of new drug agents with improved safety and efficacy [17]. Now days many scientist and organizations are searches for traditional remedies which used as alternative medicine. It has been predicted that about 25% of all prescribed medicines in which present substances are derived from plants [18]. In health care system, it has estimated more than 7000 different compounds from plant were used to make antibiotics. The world health organization (WHO) approximated that more than 80% of the world population used plants for their primary health care while since western pharmaceuticals are expensive, unsuitable, accessibly and are always accompanied with numerous side effects [18].

Table 1: Phytochemical constituents present in Methanol, Petroleum ether and Chloroform extracts of stem bark and fruit *Limonia acidissima L.*

S.No.	Phytochemical Constituent	Methanol extract		Petroleum ether extract		Chloroform extract	
		Stem bark	Fruits	Stem bark	Fruits	Stem bark	Fruits
1.	Alkaloids	+	+	-	-	+	+
2.	Tannins	-	-	+	+	-	-
3.	Flavonoids	+	+	+	+	-	+
4.	Terpenoids	-	+	+	+	-	-
5.	Steroids	+	+	-	-	+	-
6.	Saponins	+	+	-	-	-	+
7.	Phenolic compounds	+	+	-	-	+	-
8.	Glycosides	+	+	-	+	+	+
9.	Carbohydrates	+	+	-	-	+	-

(+) = Present, (-) = Absent

4. CONCLUSION

Various parts of *Limonia acidissima* are used as for treatment of human ailments. The qualitative phyto-chemical analysis work was carried out to analyze out of the bioactive compound present in the different parts of plant. The present study clearly indicated that the compounds like alkaloids, tannins, flavonoides, terpenoids, steroids, glycosides and saponins are the active principles all is present in the leaves and fruit pulp of *Limonia acidissima*.

5. ACKNOWLEDGEMENTS

We are very thankful to our respected Professor Dr. R.K. Tenguria, Department of Botany, Government Motilal Vigyan Mahavidyalaya Bhopal (M.P.) and to the Head of the Department of Biotechnology & Bio-informatics center, Barakatullah University, Bhopal (M.P.) for providing guidance and valuable suggestions for the completion of the experimental analysis.

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