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PHYTOCHEMICAL INVESTIGATION AND HPTLC FINGERPRINTING OF ANNONA SQUAMOSA, AEGLE MARMELOS LEAVES

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Abstract

The present communication attempts to investigate pharmacological, physicochemical analysis and chromatoghraphic profile of ethanol extract of *Annona squamosa* and *Aegle marmelos* leaves. This work showed the soxhelt extraction process, phytochemical screening, High Performance Thin Layer Chromatoghraphy and therapeutic importance of the medicinal herbs. Aim and objective: The objective of study is to investigate the preliminary screening of active constituents present in the ethanolic extract of leaves of *Annona squamosa* and *Aegle marmelos*. The phytochemicals was extracted separately with distilled water and 96% ethanol by soxhlet extraction method. The present study includes pharmacognostic test detection of alkaloids, glycosides, phenols, carbohydrates, saponins, reducing sugar, flavonoids, tannins. A wide variety of pharmacologically active compounds presented in Annona squamosa leaf extract alkaloids, glycosides, carbohydrates, phenols and saponins. were found to present in the leaves of *Annona squamosa* and *Aegle marmelos*. Methods: High performance thin layer chromatography, soxhlet extraction. HPTLC profile of ethanolic extact of herbs had been studied. The generated dada has provided the basis for wide uses as a therapeutic in the traditional and folk medicines. Results: The Rf values of ethanolic extract of *Annona squamosa* leaf extract run under Chloroform: Methanol(8:2) solvent system were obtained 0.10, 0.21, 0.36, 0.38, 0.42, 0.45, 0.53,0.60, 0.81, 0.86. The Rf values of ethanolic extract of *Aegle marmelos* leaf extract run under Petrolium Ether : Ethyl Acetate (2:1) solvent system were obtained 0.37,0.44,0.54.

Keyword: Extraction, HPTLC fingerprinting, Annona squamosa, Aegle marmelos.

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Introduction

In India medicinal plants which are originated from the nature have been used in tradicinal system of medicine such as Ayurveda, Unani, Siddha.The herbal medicinal plants having playing continuously is an important role in our health care system. These are medicinal herbs are reported to have significant different pharmacological activities. The growing recognition of natural and herbal medications, easy availability of raw materials, cost-effectiveness and the paucity of reported adverse reaction.The therapeutic use of many indigenous plants, for various health disease has been described by traditional herbal meditional practitioners. Natural products having many more popular in many topics of scientific researches due to there presence chemical composition the resent study watch carried out to evaluate phytochemical screening, HPTLC of *Annona squamosa* and *Aegle marmelos* (Soni Himesh et al 2011).

Materials And Methods

The plants were selected on the basis of their pharmacological activities and their medicinal uses reported in the literature. The herbs (*Annona squamosa* and *Aegle marmelos*) were purchased from the Herbal garden of P.Wadhawani College Of Pharmacy,Yavatmal and authenticated by Taxonomist in the department of Botany, Shri Shivaji Science and Arts College, Chikhli. Dist. Buldana SGBAU University (MS) All other chemicals were of analytical grade and used without further purification.

Preparation of extract

The powdered of *Annona squamosa* and *Aegle marmelos* were used for extraction. The powder is extracted in soxhlet apparatus with ethanol. The extraction procedure were carried out till a sufficient quantity of extract was obtained. The solvent was removed by distillation method(Ruchi Sharma et al 2012).

Instrument

Camag HPTLC system, Consisting of Linomat V spotting device and win cats planner chromatography manager by anchrom all tracs wavelength Sc 4 from lab of B.R. Nahata College of Pharmacy, Indore.

Stationary phase

TLC Aluminium sheets, silica gel 60 F 254 pre-coated layer (20.0X10.0cm),thickness 0.2mm,band length 6.0mm for *Aegle marmelos*. And also *Annona squamosa* have TLC Aluminium sheets, silica gel 60 F 254 pre-coated layer (10.0X10.0cm),thickness 0.2mm,band length 6.0mm.

Mobile phase

Aegle marmelos petroleum ether : ethyle acetate (2 : 1) Executed by anchrom plates 20.0 x 10.0 cm, Material HPTLC plates silica gel 60 F 254 distance run :75.0 mm, scanning wavelength : 254nm, slit dimension 4.00 x 0.30 mm, Micro Measurement Mode –Absorption.

Annona squqmosa chloroform : methanol (8 : 2) Executed by anchrom plates 10.0 x 10.0 cm, Material HPTLC plates silica gel 60 F 254 distance run :75.0 mm, scanning wavelength : 200nm, slit dimension 4.00 x 0.30 mm, Micro Measurement Mode –Absorption.

Phytochemical analysis of crude extract

Phytochemical screening of active constituents of plant extract was done by following the standard method of qualitative analysis of phytochemical study such as alkaloids, glycosides, saponin, tannin,phenols,steroids, flavonoids etc.(Khandelwal et al 2007).

Test for carbohydrates

Molisch test:- To take 1ml of extract treated with the 2-3 drop of Molish reagent (10% of 1-napthol in ethanol). Test tube taken as an angle and add 1-2 ml conc. H_2SO_4 carefully observed for the formation of reddish violet ring ring formation at the junction.

Keller-Killani Test :- Weigh about 0.5 gm of plant extract in a separate test tube with 2 ml of glacial acetic acid containing a drop of ferric chloride solution. This was under layered with 1 ml of concentrated tetra oxo sulphate acid. And observe for brown ring formation at the interface.

Test for reducing sugars:- To take 2-3ml of Fehling solution A and B were heated gently and allowed to cool. Then 1ml of extract was added to it. The mixture was boiled for 5-10 minutes. Brownish red precipitates indicated the presence of reducing sugars.

Test for Saponins :-To take about 0.2 gm of plant extract in the test tube and addition of 5 ml of distilled water and then heat to boil. Observe for the occurrence of frothing (appearance of creamy mass of small bubbles) which then indicates the presence of Saponin.

Test for Tannin:- To small quantity of plant extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. And observe for dark green solutions that indicate the presence of a tannin.

Test for Flavonoids:- Weigh about 0.2 gm plant extract in separate test tube and dissolved diluted

Sodium hydroxide and add diluted Hydrochloride. And observe for yellow solutions that turn colorless. This indicates the presence of flavonoids(Gorden MC et al 2001).

Test for Steroids:- To the plant extract add 2 ml of acetic anhydride and add 0.5 gm of ethanolic extract of each sample with 2 ml of Sulphuric acid. Observe for the color change from violet to blue or green in samples indicating the presence of steroids.

Test for alkaloids:-

Mayer's reagent test:- 1ml of 1% HCl was added to 3ml of extract in a test tube. The mixture was heated gently for 20 minutes, allowed to cool and filtered. After this, two drops of Mayer's reagent was mixed in 1ml of filtrate and observed for turbidity or creamy precipitates.

Test for Phenol

FeCl3 test:- 2-3ml of extract was treated with few drops of 10% aqueous FeCl3 and observed for the emergence of blue green colour.(Kokate CK et al 2004).

HPTLC study of *Annona squamosa* and *Aegle marmelos* leaves extract

The HPTLC analysis was performed using win CATS Planar chromatography manager anchrom. In which HPTLC plate silica gel 60 F200.The mobile phase consists of solvent mixture chloroform:methanol(8:2).Cliberation mode give single level and light optimize optical system are used. Scanning speed 20mm/s data resolution 100micro meter/step. The procedure conducted on CATMAG TLC scanner. After phytochemical analysis of ethanolic extract were subjected to HPTLC coloum and obtained record were superimposed on the retention time values of these extract.

Result

The curative properties of medicinal plants are perhaps due to the presence of various active constituents such as alkaloids, glycosides, saponnin, tannins, phenols, flavonoids.The successfully extraction process carried out of leaves of Annona squamosa and Aegle marmelos. In HPTLC report showed that the different acive constituents are present in the ethanolic extract of leaves of Annona squamosa and Aegle marmelos. Graphical peak value are shows the presence of phytochemicals in wavelength 254nm and 200nm.in both extract. Different peak value of retention factor is indicated to phytoconstituents. In all over the preliminary phytochemical screening and detection of phytoconstituents by HPTLC method was done by successfully. The Rf values of ethanolic extract of *Annona squamosa* run under Chloroform: Methanol(8:2) solvent system were 0.10, 0.21, 0.36, 0.38, 0.42, 0.45, 0.53,0.60, 0.81, 0.86. The Rf values of ethanolic extract of Aegle marmelos run under Petrolium Ether : Ethyle Acetate (2:1) solvent system were 0.37,0.44,0.54. In Annona squamosa leaves ethanolic extract presence of alkaloids,tannins, flavonois, phenols, carbohydrates And Aegle marmelos leaves ethanolic extract presence of saponin,tannins, steroids. Futher study in future isolation and separation of the phytoconstituents in the extract.

Conclusion

The present study signifies the preliminary phytochemical screening of extract and enlist all phytoconstituents present in the medicinal plants. This ancient plants are economically, medicinally and environmentally imperative. Broad spectrum of biological and pharmacological activites is reported from several parts of the tree. In all over the preliminary phytochemical screening and detection of phytoconstituents by HPTLC method was done by successfully. In Annona squamosa leaves ethanolic extract presence of alkaloids,tannins, flavonois, phenols, carbohydrates And Aegle marmelos leaves ethanolic extract presence of saponin,tannins, steroids. Futher study in future isolation and separation of the phytoconstituents in the extract.This study will be useful to research community to contribute in developing systematically validated herbal products from parts of this trees.

Data availability statement

All data analyzed during this study are included in this article.

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Competing interests

The authors declare no conflict of interest with this research.

Ethical approval

Since no animals ware used in this study, ethical approval was not needed.

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Fig.1 Annona squamosa

Fig.2 Aegle marmelos

Table 1: Preliminary Phytochemical screening of active constituents in leaves extract

No	Active constituents/ Phytochemicals and testing methods	*Annona squamosa*	*Aegle marmelos*
1	Test for Carbohydrates: Molisch test Keller-Killani Test	+	-
2	Test for reducing sugars	-	-
3	Test for Saponins Foam test Haemolytic test	-	+
4	Test for Tannin	+	+
5	Test for Flavonoids Shinoda test	+	-
6	Test for Steroids	-	+
7	Test for alkaloids Mayers test Wagners test	 + +	- -
8	Test for Phenol FeCl3 test	+	-

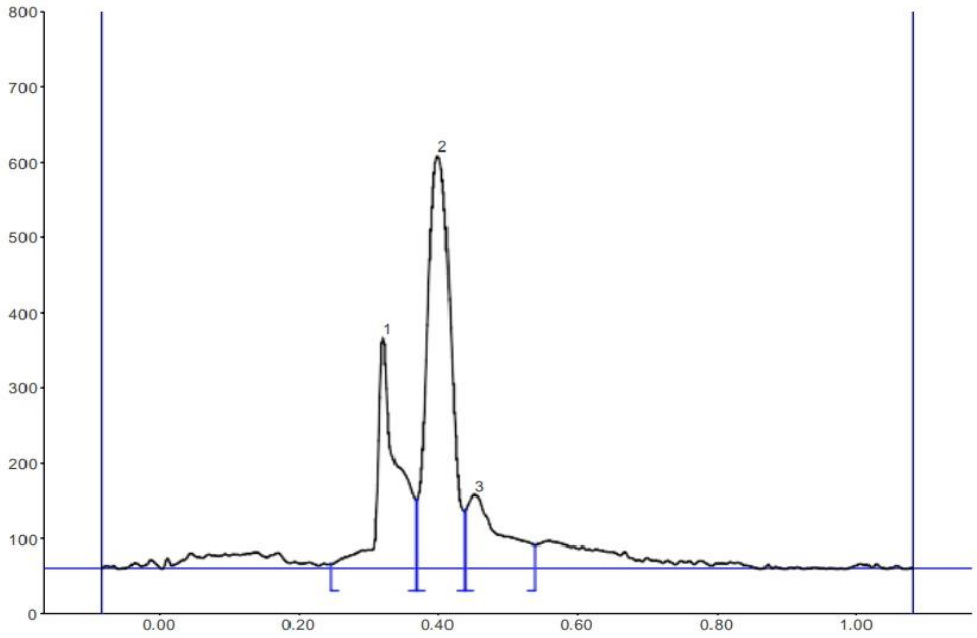
+ Positive test and – Negative test

Table 2: Peak value obtained by Aegle marmelos leaf extract

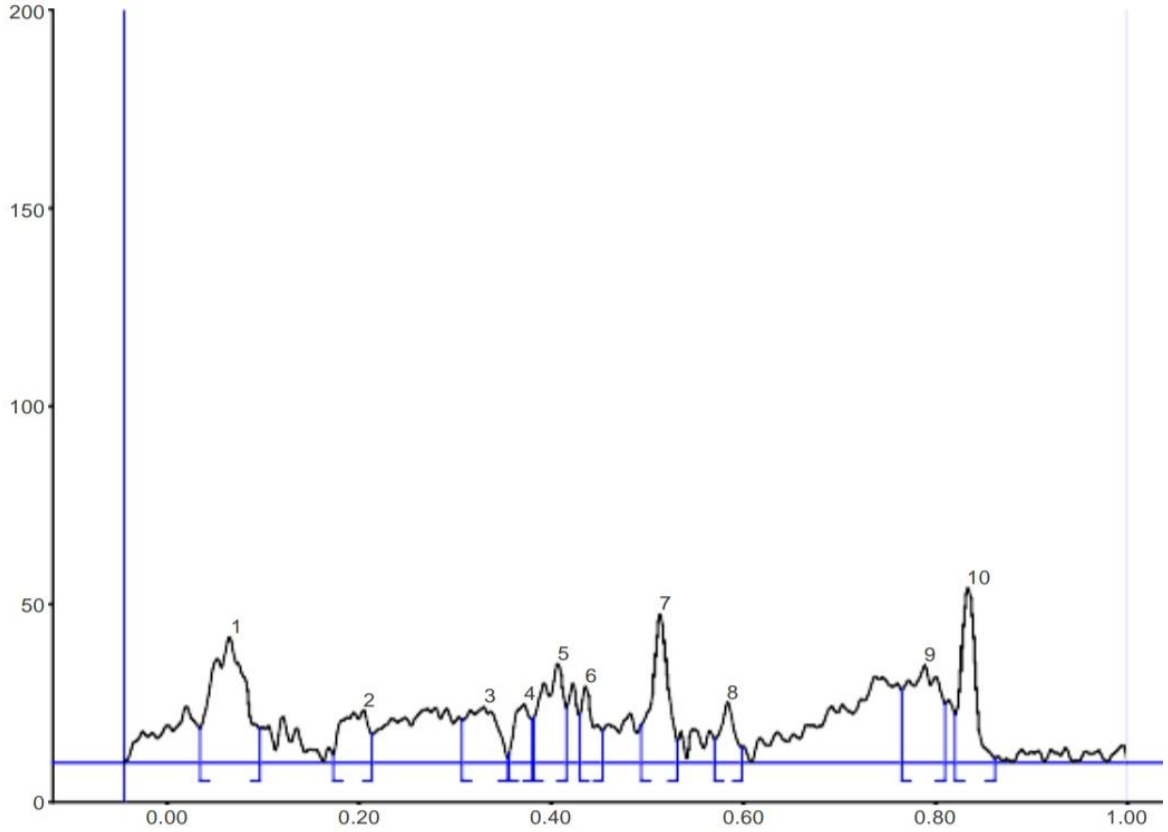
Peak	Start Rf	Start Height	Mx Rf	Max Height	Max %	End Rf	End Heigt	Area	Area %
1	0.25	7.2	0.32	306.6	32.13	0.37	92.6	6300.0	27.87
2	0.37	92.8	0.40	548.9	57.53	0.44	77.6	12857.9	56.87
3	0.44	79.0	0.45	98.6	10.34	0.54	91.6	3449.4	15.26

Table 3: Peak value obtained by Annona squamosa leaf extract

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.03	8.5	0.07	31.6	13.28	0.10	8.5	850.2	20.73
2	0.17	2.0	0.20	13.1	5.50	0.21	7.0	277.3	6.76
3	0.31	10.6	0.33	13.8	5.81	0.36	1.0	343.4	8.37
4	0.36	1.9	0.37	14.6	6.13	0.38	10.6	185.4	4.52
5	0.38	11.1	0.41	24.9	10.46	0.42	13.7	432.0	10.53
6	0.43	11.7	0.44	19.1	8.05	0.45	7.9	206.5	5.04
7	0.49	9.4	0.51	37.4	15.73	0.53	5.1	493.6	12.03
8	0.57	5.7	0.58	15.0	6.30	0.60	3.7	172.5	4.21
9	0.77	18.2	0.79	24.4	10.27	0.81	14.7	622.8	15.18
10	0.82	12.0	0.83	43.9	18.48	0.86	0.8	517.8	12.62



Graphical presentation of Rf value of Aegle marmelos leaf extract



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