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HPTLC Profiling Of *Annona Squamosa* L.

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Abstract:
Medicinal plants serve as important source of phytochemicals (secondary metabolites) which have protective, preventive and curative properties including -antibacterial, anticancer, antifungal, and antioxidant (Samidha M. Pawaskar and K. C. Sasangan 2017). The naturally occurring antioxidants in them possess the ability to reduce the oxidative damage associated with many diseases, including cancer, cardiovascular disease, cataracts, atherosclerosis, diabetes, arthritis, immune deficiency diseases and aging.n the present study HPTLC finger print scanned at wavelength 254nm for ethanol extract of Annona squamosa L. stem (six polyvalent phytoconstituents was found 38.62%), leaf (nine polyvalent phytoconstituents was found 48.55%), fruit cover (eight polyvalent phytoconstituents was found 56.64%), seed (seven polyvalent phytoconstituents was found 57.47%) similar study was carried out by Mona Agrawal et al 2012.

Key Words:*Annona squamosa L., phytochemicals, traditional uses, HPTLC analysis*

Introduction

Annona squamosa Linn. (Annonaceae) commonly known as Sitaphal(custard apple) is a small tropical tree that is native to South America. It is a shrub or tree, having height up to 8 m, trunks short, not buttressed at base. Traditionally, bark decoction is used to stop diarrhoea, while the root is used in the treatment of dysentery. A extract of the leaves is used as a cold remedy. The fruits of Annona are haematinic, cooling, sedative, stimulant, expectorant and tonic (W. Evans, 1997). They are useful in anaemia, burning sensation. The seeds are abortifacient and insecticidal and are useful in destroying lice in the hair. Scientific investigations have shown that the crude extract possesses miticidal,antifeedant, antidiabetic and anxiolytic activities (Chavan et al, 2010). Traditional medicines have been used worldwide since time immemorial. The term traditional medicine is interchangeably used with herbal medicine and natural medicine. The whole or part of medicinal plants have secondary metabolites whichuseful for therapeutic purposes and synthesis of medicine or drugs. Plants have been one of the important sources of medicines since the beginning of human civilization (Chitra Shenoy et al, 2009). There is a growing demand for plant based medicine, health products, food supplements, pharmaceutical, cosmetics.

Annona squamosa is a multipurpose indigenous plant belonging to the family Annonaceae.It contains several constituent belonging to category alkaloids, glycoside, flavonoids, Terpenoids,

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steroids and fruit pulp contain important nutrients and antimicrobial activity (Chandrashekhar and Kulkarni, 2011)

Materials and Methods

1. Collection of rind:

Different plant parts of *Annona squamosa* such as stem, leaves, fruits and seeds were collected from medicinal garden of KRA college,Deola dist. Nashik. The collected plant material was brought into laboratory for further analysis. Stem, leaves, fruit cover and seeds were dried separately. The dried plant material was ground into powder form using mixture grinder.

2. Extraction of Plant Material:

About 20 gm powder of stem, leaves, fruit cover and seeds were extracted separately using 80% ethanol in a Soxhlet Extractor (Borosil) for about six hours. After extraction the extracts were evaporated to dryness using hot plate. The dried extracts were redissolved in 5 ml ethanol and filtered using Whatmann filter. The filtered extracts were used for further phytochemical and HPTLC analysis.

3. HPTLC Analysis of Extracts: -

HPTLC fingerprinting extracts of were carried out as per the method described by Mona Agrawal et. al (2012). Two micro liters of the ethanolic extract was applied (band length –8.0 mm) on a precoated TLC aluminium sheets of silica gel G60 F254 of 200 µm thickness plate- 05 x10cm (Merck, Mumbai) using Linomat V TLC applicator (Camag, Muttenz, Switzerland) equipped with a 100-µL syringe. Before application, theplate was pre-cleaned with methanol AR and dried at 60°C. TLC plates were developed using the mobile phase Toluene:ethyl acetate:diethyl ether:methanol:chloroform (12:6:2:2:1) in a Camag HPTLC twin-trough chamber (10 x10cm). The TLC chamber was concentrated with filter paper for 15 minutes and plate equilibrium was carried out for 10 minutes. **TLC Plate was developed** up to 85.0 mm and dried under stream of air. Separated bands were quantified by HPTLC densitometric scanning using Camag TLC Scanner 4 in the absorption mode (multi wavelength Scanning) operated by Win CATS software (version 1.4.8). After scanning the spectra and tables thus obtained were analyzed to interpret the results.

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Fig 1. *Annona squamosa plant*

Fig 2. Fruit -*Annona squamosa*

Fig. 3. Seeds

Result and discussion:

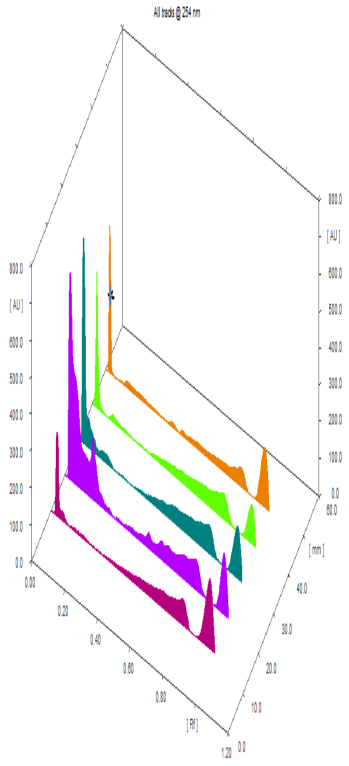

Fig 1.3 D Spectral display of stem, leaves,

HPTLC plate Scanned at 356nm

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fruit coatand seed of *Annona squamosa*

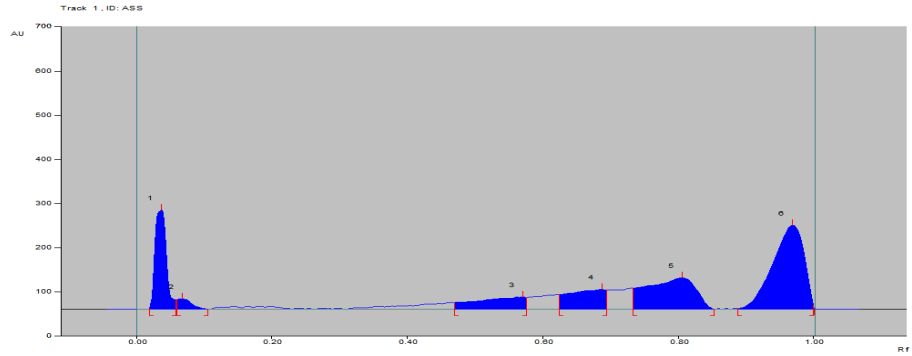

Fig 2: HPTLC profile (Peak Display) of *Annona squamosa* stem

Track 1, ID: ASS

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	1.2 AU	0.04 Rf	224.0 AU	38.62 %	0.06 Rf	21.3 AU	3222.7 AU	17.08 %
2	0.06 Rf	21.5 AU	0.07 Rf	23.2 AU	4.00 %	0.11 Rf	0.0 AU	406.6 AU	2.15 %
3	0.47 Rf	15.1 AU	0.57 Rf	27.7 AU	4.77 %	0.58 Rf	26.3 AU	1672.4 AU	8.86 %
4	0.63 Rf	32.8 AU	0.69 Rf	44.7 AU	7.71 %	0.70 Rf	43.4 AU	2025.1 AU	10.73 %
5	0.73 Rf	47.2 AU	0.81 Rf	70.6 AU	12.17 %	0.85 Rf	0.1 AU	4300.6 AU	22.79 %
6	0.89 Rf	1.4 AU	0.97 Rf	189.9 AU	32.73 %	1.00 Rf	8.6 AU	7245.1 AU	38.39 %

Fig 3: HPTLC profile (Peak Table) of *Annona squamosa* stem

The results from HPTLC finger print scanned at wavelength 254 nm for ethanol extract of *Annona squamosa* stem showed six polyvalent phytoconstituents and corresponding ascending order of Rf values start from 0.04 to 0.97 in which highest conc. of the phytoconstituents was found to be 38.62 % and its corresponding Rf value was found to be 0.04 respectively. This is recorded in Figure 3. The corresponding HPTLC chromatogram was presented in Figure 2 which shows six peaks of phytoconstituents

Fig 4: HPTLC profile (Peak Display) of *Annona squamosa* leaf

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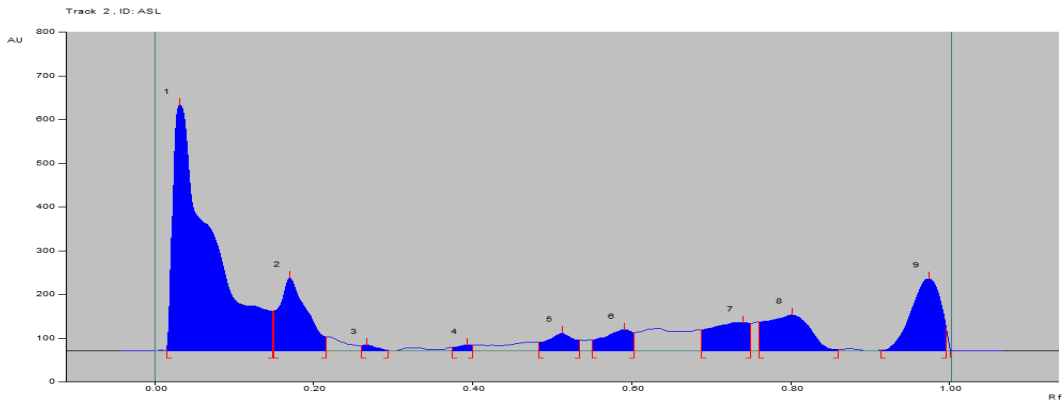

Fig 5: HPTLC profile (Peak Table) of *Annona squamosa* leaf

Track 2, ID: ASL

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	3.1 AU	0.03 Rf	561.5 AU	48.55 %	0.15 Rf	91.3 AU	22076.2 AU	53.41 %
2	0.15 Rf	91.5 AU	0.17 Rf	167.1 AU	14.45 %	0.22 Rf	32.2 AU	4749.0 AU	11.49 %
3	0.26 Rf	12.0 AU	0.27 Rf	13.1 AU	1.14 %	0.29 Rf	1.0 AU	206.0 AU	0.50 %
4	0.38 Rf	7.5 AU	0.39 Rf	14.0 AU	1.21 %	0.40 Rf	12.8 AU	222.2 AU	0.54 %
5	0.48 Rf	19.0 AU	0.51 Rf	40.6 AU	3.51 %	0.54 Rf	24.1 AU	1113.6 AU	2.69 %
6	0.55 Rf	25.3 AU	0.59 Rf	48.5 AU	4.19 %	0.60 Rf	41.8 AU	1474.3 AU	3.57 %
7	0.69 Rf	48.0 AU	0.74 Rf	65.0 AU	5.62 %	0.75 Rf	62.7 AU	2695.7 AU	6.52 %
8	0.76 Rf	65.5 AU	0.80 Rf	82.0 AU	7.09 %	0.86 Rf	2.7 AU	3827.9 AU	9.26 %
9	0.92 Rf	0.9 AU	0.98 Rf	164.6 AU	14.24 %	1.00 Rf	53.6 AU	4972.2 AU	12.03 %

The results from HPTLC finger print scanned at wavelength 254 nm for ethanol extract of *Annona squamosa* leaf showed nine polyvalent phytoconstituents and corresponding ascending order of Rf values are from 0.03 and 0.98 in which highest conc. of the phytoconstituents was found to be 48.55 % and its corresponding Rf value was found to be 0.03 respectively. This is recorded in Figure 5. The corresponding HPTLC chromatogram was presented in Figure 4 which shows nine peaks of phytoconstituents

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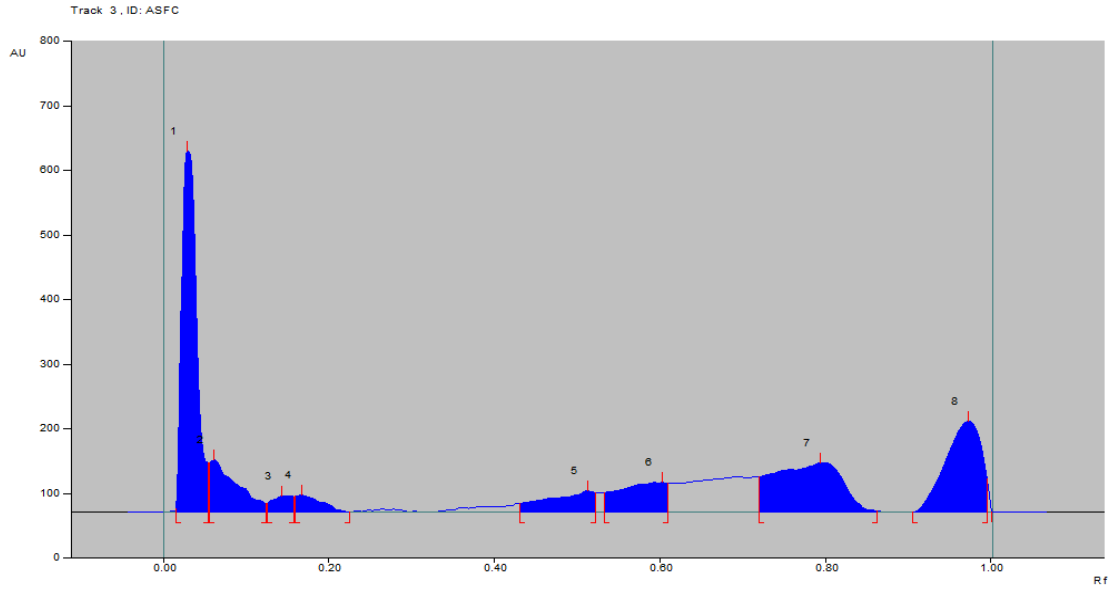

Fig 6: HPTLC profile (Peak Display) of *Annona squamosa* fruit cover

Track 3, ID: ASFC

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	5.0 AU	0.03 Rf	558.8 AU	56.64 %	0.06 Rf	76.4 AU	8393.7 AU	32.42 %
2	0.06 Rf	76.5 AU	0.06 Rf	80.9 AU	8.20 %	0.13 Rf	13.3 AU	2204.0 AU	8.51 %
3	0.13 Rf	13.8 AU	0.14 Rf	24.8 AU	2.51 %	0.16 Rf	24.0 AU	519.9 AU	2.01 %
4	0.16 Rf	24.1 AU	0.17 Rf	26.4 AU	2.68 %	0.23 Rf	0.1 AU	688.7 AU	2.66 %
5	0.43 Rf	13.5 AU	0.51 Rf	33.1 AU	3.36 %	0.52 Rf	30.5 AU	1512.5 AU	5.84 %
6	0.53 Rf	30.1 AU	0.60 Rf	45.7 AU	4.64 %	0.61 Rf	44.5 AU	2220.2 AU	8.58 %
7	0.72 Rf	54.9 AU	0.80 Rf	76.6 AU	7.77 %	0.86 Rf	1.1 AU	5334.4 AU	20.60 %
8	0.91 Rf	0.1 AU	0.97 Rf	140.2 AU	14.21 %	1.00 Rf	50.5 AU	5017.1 AU	19.38 %

Fig 7: HPTLC profile (Peak Table) of *Annona squamosa* fruit cover

The results from HPTLC finger print scanned at wavelength 254 nm for ethanol extract of *Annona squamosa* fruit cover showed eight polyvalent phytoconstituents and corresponding ascending order of Rf values are from 0.03 to 0.97 in which highest conc. of the phytoconstituents was found to be 56.64 % and its corresponding Rf value was found to be 0.03 respectively. This is recorded in Figure 6. The corresponding HPTLC chromatogram was presented in Figure 7 which shows eight peaks of phytoconstituents

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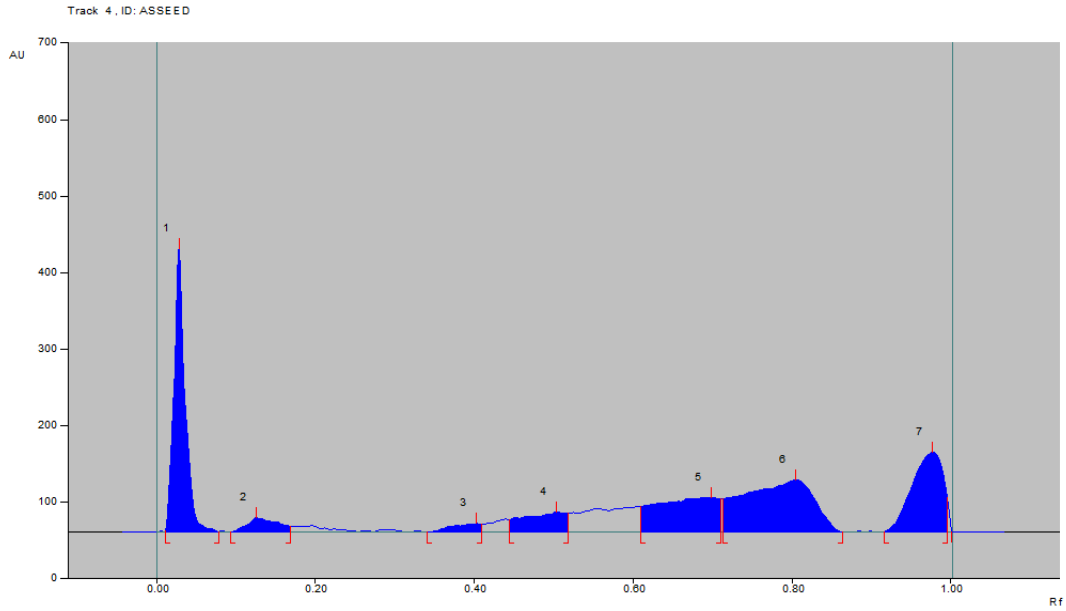

Fig 8: HPTLC Peak Display of *Annona squamosa* seed

Track 4, ID: ASSEED

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	1.5 AU	0.03 Rf	370.0 AU	57.47 %	0.08 Rf	0.3 AU	4270.3 AU	24.02 %
2	0.09 Rf	0.1 AU	0.13 Rf	18.5 AU	2.88 %	0.17 Rf	7.4 AU	591.5 AU	3.33 %
3	0.34 Rf	0.4 AU	0.40 Rf	11.0 AU	1.70 %	0.41 Rf	9.5 AU	334.0 AU	1.88 %
4	0.45 Rf	15.8 AU	0.50 Rf	26.1 AU	4.06 %	0.52 Rf	24.1 AU	1151.3 AU	6.48 %
5	0.61 Rf	33.2 AU	0.70 Rf	45.5 AU	7.06 %	0.71 Rf	43.5 AU	2984.9 AU	16.79 %
6	0.71 Rf	43.5 AU	0.81 Rf	68.1 AU	10.57 %	0.87 Rf	0.0 AU	5071.5 AU	28.52 %
7	0.92 Rf	0.3 AU	0.98 Rf	104.7 AU	16.26 %	1.00 Rf	45.0 AU	3377.5 AU	18.99 %

Fig 9: HPTLC Peak Table of *Annona squamosa* seed

The results from HPTLC finger print scanned at wavelength 254 nm for ethanol extract of *Annona squamosa* seed showed seven polyvalent phytoconstituents and corresponding ascending order of Rf values are from 0.03 to 0.98 in which highest conc. of the phytoconstituents was found to be 57.47 % and its corresponding Rf value was found to be 0.03 respectively. This is recorded in Figure 9. The corresponding HPTLC chromatogram was presented in Figure 8 which shows seven peaks of phytoconstituents

Annona squamosa L plant consists of wide variety of novel biochemical's which were used in antibacterial, anticancer, antifungal, antidiabetic, pesticidal and antioxidant

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