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Occurrence of Bioactive compounds in *Ananus comosus* (L.): A quality Standardization by HPTLC

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ABSTRACT

Objective: *Ananus comosus* (L.) is belonging to the family Bromeliaceae used to cure various diseases. The present study is focus to evaluate the HPTLC fingerprinting analysis and phytochemical analysis of *Ananus comosus* peel. **Methods:** HPTLC fingerprinting profiles was done by using Hamilton syringe and CAMAG LINOMAT 5 instrument. **Results:** Among all solvents, most of the secondary metabolites such as alkaloid, flavanoid, phenol, tannins, steroids etc were present in ethanolic extract of *Ananus comosus* peel and the yield of plant extract in different solvents were analyzed, from which ethanolic extract gave 2.40g/100g. The quantitative assay was also analyzed in flavanoid, alkaloid, phenols, carotenoid and lycopene. HPTLC fingerprinting profile confirms the presence of phenols, flavanoid and alkaloid. **Conclusion:** From the above results ethanolic extract of *Ananus comosus* peel can be used as therapeutic agents to treat various disorders caused by free radical and chemical substances due to presence of its secondary metabolites.

1. Introduction

Medicinal plants are the local heritage with global importance world is endowed with a rich wealth of medicinal plants. These plants have made a good contribution to the development of ancient materia medica[1]. They are an important therapeutic aid for various dreadful diseases. In India, from ancient times, different parts of medicinal plants have been used to cure specific diseases. Herbs have been used as sources of food and medicinal purposes for centuries and knowledge has been passed on from generation to generation and also have good antioxidant properties without any side effects[2].

Plants are widely in the traditional medicine and their curative potentials are well documented. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. The inhabitants of an area utilize plant wealth for medicine, food and many other purposes. Many of the food and medicinal plants contain a variety of chemical substances such as alkaloids, tannins, flavonoids, steroids, glucosides, saponins, oxalates, etc. Thus

phytochemical screening of such plants is an important aspect for the scientific verification of folklore claim with regard to the utility of plants[3].

Ananus comosus (L.) is belonging to the family Bromeliaceae is used in folk remedies for digestive disorder and diuretic property. Juice of the leaves consumed for hiccoughs and vermifuge. Juice of ripe fruit regarded also as antiscorbutic, cholagogic, diaphoretic, refrigerant, and useful in jaundice. Young vegetative buds are used for respiratory ailments among Choco children. The enzyme complex of *A. comosus* called bromelain is known for its clinical applications particularly modulation of tumor growth, blood coagulation and anti-inflammatory effect[4]. The enzyme complex of *A. comosus* called bromelain is known for its clinical applications particularly modulation of tumor growth, blood coagulation, anti-inflammatory effect etc[5]. The present study was aimed to evaluate the HPTLC fingerprinting profile and phytochemical analysis of *Ananus comosus* (L.) Merr peel.

2. Materials and methods

2.1 Collection of Plant material

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Fresh pineapple peel was collected from Coimbatore, Tamil Nadu, India. The plant was authenticated by Dr. G.V.S Moorthy, Botanical survey of India, TNAU Campus, Coimbatore and the voucher specimen No.BSI/SRC/5/23/2011/Tech-515. Fresh peel sample was washed under running tap water, air dried, and then homogenized to fine powder and stored in airtight bottles.

2.2 Sample Extraction

100g of dried plant powder was extracted in 500ml of ethanol in a water shaker for 72hrs. Repeatedly extraction was done with the same solvent till clear colorless solvent is obtained. Obtained extract was evaporated to dryness by using a rotary vacuum evaporator at 40–50°C and stored at 0–4°C in an air tight container.

2.3 Phytochemical analysis

2.3.1 Qualitative method

Preliminary phytochemical screening of the ethanolic extract of *Ananus comosus* was estimated according to the method[6].

2.3.2 Quantitative method

2.3.2.1 Estimation of total phenolic content

The total phenolic in extract was determined according to Folin–Ciocalteu procedure by Singleton and Rossi[7]. Four hundred microlitres of sample (two replicates) were taken in test tubes; 1.0ml of Folin–Ciocalteu reagent (diluted 10 fold with distilled water) and 0.8ml of 7.5% sodium carbonate were added. The tubes were mixed and allowed to stand for 30min and the absorption at 765nm was measured against blank, which contained 400 μ l of ethanol in place of sample. The total phenolic content was expressed as catechol equivalents in mg g⁻¹ of ethanolic extract.

2.3.2.2 Estimation of total flavonoid content

The total flavonoid content of the pineapple peel extract was determined using a modified colorimetric method[8]. Briefly, 0.25ml of extract was mixed with 1.25ml of distilled water and subsequently with 0.075ml of 5% sodium nitrite solution and was allowed to react for 5 min. Then a 0.15ml of 10% aluminium chloride was added and allowed to further react for 6 min before 0.5ml of 1M sodium hydroxide was added. Distilled water was added to bring the final volume of the mixture to 3ml. The absorbance of the mixture was immediately measured at a 510 nm wavelength against a prepared blank. The flavonoid content was determined by a catechin standard curve and expressed as the mean (milligrams of catechin equivalents per gram of fresh sample) \pm SD for the triplicate extracts.

2.3.2.3 Estimation of Tannins

The tannin content was estimated by Vanillin–HCL method[9]. To 0.5 ml of sample, 0.5 ml of distilled water and 4.0ml of Vanillin–HCL were added and mixed thoroughly. The absorbance of all samples was measured at 500 nm using a spectrophotometer. From the standard graph the amount of tannin present in the sample was calculated.

2.3.2.4 Estimation of Carotene and Lycopene

Carotene and Lycopene were estimated by according to the method given by[10].

2.4 HPTLC fingerprinting analysis

2 μ l of the above test solution and 2 μ l of standard solution were loaded as 5mm band length in the 3 x 10 Silica gel 60F254 TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phases (Alkaloid, Flavonoids and Phenols) and the plate was developed in the respective mobile phase up to 90mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo–documentation chamber (CAMAG REPROSTAR 3) and captured the images at White light, UV 254nm and UV366nm. The developed plate was sprayed with respective spray reagent (Alkaloid) and dried at 100°C in Hot air oven. The plate was photo–documented at Daylight and UV 366nm mode using Photo–documentation (CAMAG REPROSTAR 3) chamber. After derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at 500nm. The Peak table, Peak display and Peak densitogram were noted[11].

3. Results

In the present work, ethanolic extract of *Ananus comosus* peel were used to analyze the phytochemical constituents, percentage of yield, bioactive components and test for HPTLC analysis. Preliminary phytochemical analysis and percentage of yield in different solvent extracts were studied and the results were depicted in table 1 and 2. The ethanolic extract of *Ananus comosus* peel revealed the presence of alkaloid, flavanoid, phenol, tannins, steroids etc and the percentage of yield in plant extract were found to be 2.40g/100g.

The results of quantitative estimation of flavonoids, phenols, tannins, carotenoid and lycopene are tabulated in Table 3. The total flavonoid content of the ethanolic peel extract was 11.20 \pm 0.23mg of catechin/g extract. The total phenolic content of the plant were 120.87 \pm 0.15 mg of catechol/g extract. The total tannin content of the extract were found to be 4.09 \pm 0.12mg of catechin/g extract and the total carotenoid and lycopene content of the peel extract were possessed 22.39 \pm 0.44mg/g and 20.36 \pm 0.12mg/100g. These

phytochemical compounds are known to support bioactive activities in medicinal plants and thus responsible for the antioxidant activities of this plant extract.

3.1 HPTLC fingerprinting analysis

HPTLC profile of ethanolic extract was generated in solvent systems of different polarities in order to ascertain the total number of chemical moieties which will also help in designing the method of isolation and characterization of bioactive compounds.

Table 2

% of Yield in different solvent of *Ananus comosus* Peel

Solvent	% of Yield (g/100g)
Petroleum ether	1.20
Chloroform	1.93
Ethylacetate	1.03
Ethanol	2.40
Aqueous	2.12

Table 3

Bioactive Compounds of Ethanolic extract of *Ananus comosus* Peel

Bioactive compounds	Ethanolic extract
Total Flavonoid (mg/g)a	11.20±0.23
Total Phenols (mg/g)b	120.87±0.15
Tannins (mg/g)a	4.09±0.12
Total Carotenoids (mg/g)	20.36±0.12
Lycopene (mg/100g)	22.39±0.44

Values are expressed as mean ±SD (n = 3).
amg of catechin/g bmg of catechol/g

HPTLC profile (Phenols) of ethanolic extract of *Ananus comosus* peel was recorded in Table 4 and Figure 1, 2. Blue coloured zones at Day light mode present in the given standard and sample track observed in the chromatogram after derivatization, which confirmed the presence of phenols in the given standard and in the sample.

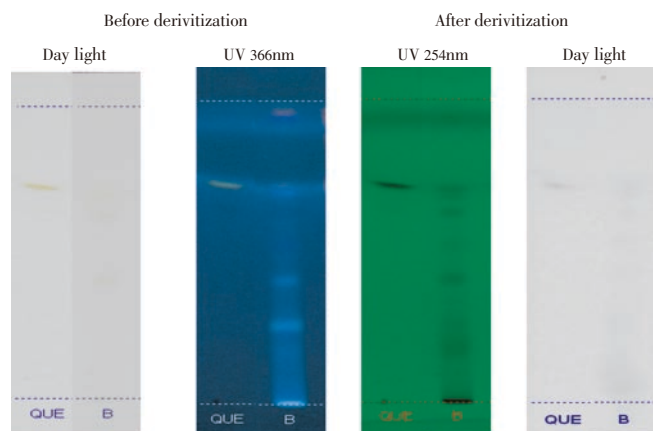


Fig 1: Chromatograms of extract in HPTLC analysis –Before derivatization: under day light, under UV 366 nm, under UV 254 nm, After derivatization: under day light mode

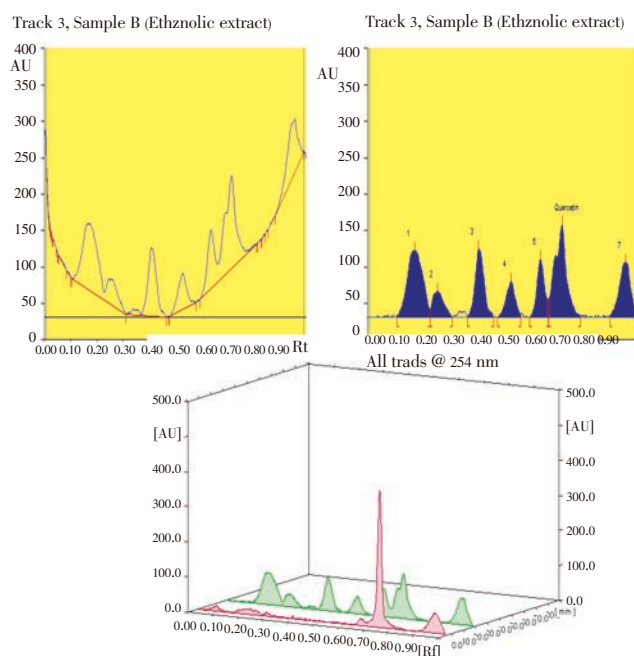


Figure.2 : Densitogram, Baseline and 3D display for phenols of ethanolic extract of *Ananus comosus*

HPTLC chromatogram of ethanolic extract of *Ananus comosus* at 254nm, showing different peaks (bands) of phytoconstituents in which 6th peak was found to be quercetin with Rf value of 0.73. Concentration of sample 100mg/ml. Toluene–Acetone–Formic acid (4.5: 4.5: 1).

HPTLC profile (Flavanoid) of ethanolic extract of *Ananus comosus* peel was recorded in Table 5 and Figure 3, 4. Yellow coloured fluorescent zone at UV 366nm mode present in the given standard track observed in the chromatogram after derivatization, which confirmed the presence of flavanoid in the given standard and may not be in the sample.

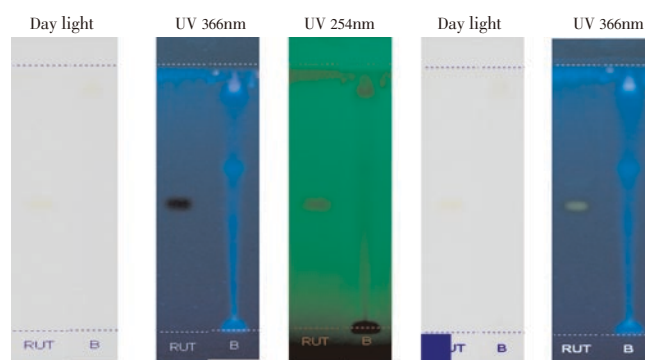


Figure 3. HPTLC fingerprinting profile (Flavanoid)
Chromatograms of sample extract (100mg/ml) in HPTLC analysis –Before derivatization: under day light, under UV 366nm, under UV 254nm, After derivatization: under day light and UV 366 nm

Table1

Phytochemical screening of *Ananus comosus* Peel

Secondary metabolites	Petroleum ether	Chloroform	Ethylacetate	Ethanol	Aqueous
Alkaloid	–	+	–	+	–
Carbohydrate and Glycosides	–	–	+	+	–
Protein and Aminoacids	–	–	–	+	–
Phenols	+	–	–	+	+
Flavonoids	–	–	–	+	–
Phytosterols	–	–	–	+	–
Steroids	–	+	–	+	–
Saponins	–	–	+	+	–
Tanins	–	–	–	+	–
Terpenoid	–	–	–	+	–
Resins	–	–	–	+	–
Oils	–	–	–	+	–

Note: “+”– Presence of secondary metabolites; “–”– Absence of secondary metabolites

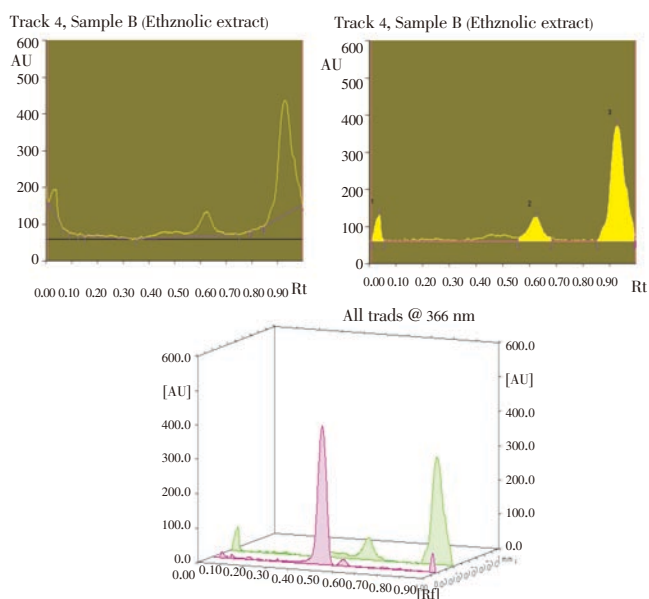


Figure 4: Densitogram, Baseline and 3D display for Flavanoid of ethanolic extract of *Ananus comosus*

HPTLC chromatogram of ethanolic extract at 366nm, showing different peaks (bands) of phytoconstituents in *Ananus comosus*. Concentration of sample 100mg/ml. Ethyl acetate–Butanone–Formic acid–Water (5 : 3 : 1 : 1)

HPTLC profile (Alkaloid) of ethanolic extract of *Ananus comosus* peel was recorded in Table 6 and Figure 5, 6.

Table 4

Peak table with Rf values, height and area of Phenols

Track	Peak	Rf	Height	Area	Assigned substance
QUE	1	0.73	392.4	7334.5	Quercetin standard
Sample B	1	0.17	92.0	4911.3	Phenolic 1
Sample B	2	0.26	35.9	1357.3	Phenolic 2
Sample B	3	0.41	94.0	2847.1	Phenolic 3
Sample B	4	0.53	49.4	1526.9	Phenolic 4
Sample B	5	0.64	79.8	2109.4	Phenolic 5
Sample B	6	0.73	126.6	4974.0	Phenolic 6 (Quercetin)
Sample B	7	0.95	75.1	2934.9	Unknown

Yellow–brown coloured zones at Day light mode present in the given standard and sample track observed in the chromatogram after derivatization, which confirmed the presence of alkaloid in the given standard and in the sample.

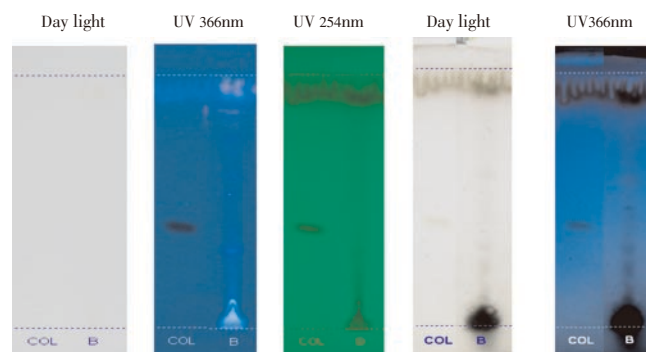


Figure 5. HPTLC fingerprinting profile (Alkaloid)

Chromatograms of extract in HPTLC analysis –Before derivatization: under day light, under UV 366nm, under UV 254nm, After derivatization: under day light and UV 366nm

Table 5

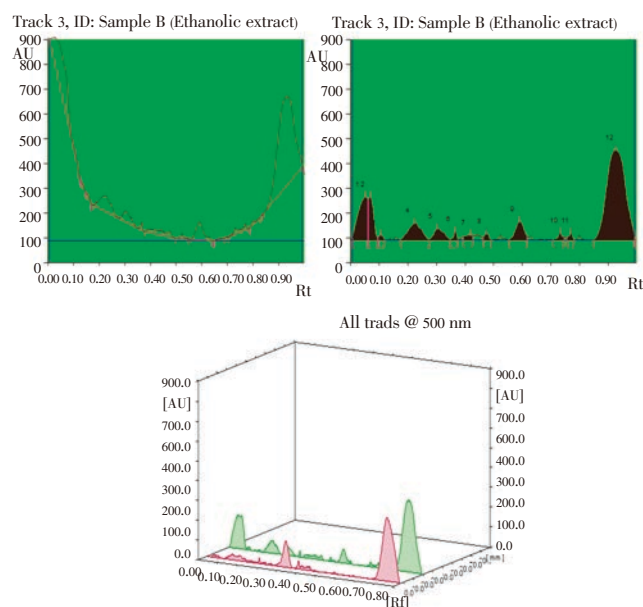
Peak table with Rf values, height and area of Flavanoids

Track	Peak	Rf	Height	Area	Assigned substance
RUT	1	0.49	403.2	14734.5	Rutin standard
Sample B	1	0.04	71.0	1339.7	Unknown
Sample B	2	0.63	65.7	3196.7	Unknown
Sample B	3	0.93	310.7	15661.6	Unknown

Table 6

Peak table with Rf values, height and area of Alkaloid

Track	Peak	Rf	Height	Area	Assigned substance
COL	1	0.41	132.8	3583.7	Colchicine standard
Sample B	1	0.05	171.6	5067.0	Alkaloid 1
Sample B	2	0.07	170.8	2554.4	Unknown
Sample B	3	0.11	17.5	229.3	Alkaloid 2
Sample B	4	0.23	65.7	2592.6	Alkaloid 3
Sample B	5	0.30	44.0	1303.7	Alkaloid 4
Sample B	6	0.37	32.1	231.6	Unknown
Sample B	7	0.42	16.9	416.3	Unknown
Sample B	8	0.48	22.3	307.6	Unknown
Sample B	9	0.59	71.5	1799.7	Alkaloid 5
Sample B	10	0.63	16.7	119.7	Unknown
Sample B	11	0.73	24.7	317.6	Unknown
Sample B	12	0.77	24.7	249.8	Unknown
Sample B	13	0.93	360.8	21016.1	Unknown

**Figure 6:** Densitogram, Baseline and 3D display for Alkaloid of ethanolic extract of *Ananus comosus*

HPTLC chromatogram of ethanolic extract at 500nm, showing different peaks (bands) of phytoconstituents in *Ananus comosus*. Concentration of sample 100mg/ml. mobile phase Ethyl acetate–Methanol–Water (10 : 1.35 : 1).

4. Discussion

Among all solvents, most of the secondary metabolites such

as alkaloid, flavanoid, phenol, tannins, steroids etc were present in ethanolic extract of *Ananus comosus* peel and the yield of plant extract in different solvents were analyzed, from which ethanolic extract gave 2.40g/100g. The medicinal plants are rich in secondary metabolites which include alkaloids, flavanoid, tannins, glycosides and related active metabolites which are great medicinal values and have been extensively used in the drug and pharmaceutical industry^[12]. These findings had confirmed with the previous reports^[13, 14]. Thus the preliminary phytochemical tests are helpful in finding chemical constituents in the plant material that may lead to their quantitative estimation and also in locating the source of pharmacologically active chemical compounds.

Flavonoids have been shown to exhibit their actions through effects on membrane permeability, and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase A₂^[15], and this property may explain the mechanisms of antioxidative action of *A. comosus*. Flavonoids serve as health promoting compound as a results of its anion radicals. These observations support the usefulness of this plant in folklore remedies in the treatment of stress-related ailments and as dressings for wounds normally encountered in circumcision rites, bruises, cuts and sores^[16].

The presence of these phenolic compounds in this plant contributed to their antioxidative properties and thus the usefulness of these plants in herbal medicament. Phenols have been found to be useful in the preparation of some antimicrobial compounds such as dettol and cresol. This plant is used routinely among many tribes in Africa for the treatment of various diseases^[17].

Tannins in this study were indicated to be present but

in low concentration in both plant parts. This bioactive compound is known to have potentials anti-viral activity as well as potential prophylactic and therapeutic effect against cancer cells, but via different mechanisms^[18].

4.1 HPTLC fingerprinting analysis

Blue coloured zones at Day light mode present in the given standard and sample track observed in the chromatogram after derivatization, which confirmed the presence of phenols in the given standard and in the sample. The extract was run along with the standard Quercetin compound. The Rf value of the peel extract was found to be 0.17, 0.26, 0.41, 0.53, 0.64, 0.73, 0.95 of peak 1, 2, 3, 4, 5, 6 and 7 respectively. Among them peaks 1, 2, 3, 4, 5 were found as phenols and the peak 6 were found to be a phenolic compound caller quercetin.

Yellow coloured fluorescent zone at UV 366nm mode present in the given standard track observed in the chromatogram after derivatization, which confirmed the presence of flavanoid in the given standard and may not be in the sample (Fig.3, 4). The extract was run along with the standard Rutin compound.

Yellow–brown coloured zones at Day light mode present in the given standard and sample track observed in the chromatogram after derivatization, which confirmed the presence of alkaloid in the given standard and in the sample (Fig.5, 6). The extract was run along with the standard Colchicine compound. The Rf value of the peel extract was found to be 0.05, 0.06, 0.11, 0.23, 0.30, 0.37, 0.42, 0.48, 0.59, 0.63, 0.73, 0.77, 0.93 of peak 1–13 respectively. Among them peaks 1, 3, 4, 5 and 9 were found as alkaloid. The above results are in accordance with the reports of^[19, 20, 21].

5. Conclusion

Based on the result in the study, we conclude that the ethanolic extracts of *Ananus comosus* peel has significant amount of secondary metabolites which might be act as a pharmacotherapeutic agent in future.

6. Acknowledgement

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7. Conflict of Interest Statement

We declare that we have no conflict of interest

8. References

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