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ORIGINAL ARTICLE **OPEN ACCESS**

Phytochemical Screening, GC-MS Analysis and Antioxidant Activity in *Ananas Comosus* Peel

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ABSTRACT

This study evaluated the phytochemical screening, gas chromatography-mass spectrometry (GC-MS) analysis and antioxidant activity of Ananas comosus using its sample with standard methods. Hence in the present study screening of Secondary metabolites from peels of Ananas comosus was carried out. Qualitative phytochemical analysis of these plants confirm the presence of various phytochemicals like alkaloids, glycosides, Flavonoids, tannins, steroids, saponins, terpenoids, steroids, anthocyanins, coumarins, and fatty acids. GC–MS analysis revealed that many useful constituents indicating Ananas comosus leaves could be useful for preparation of neutraceuticals as potent antioxidant to treat various human diseases and its complications. Some of the compounds as revealed by GC-MS analysis could be healthcare or industrial importance. This study was evaluated the phytochemical screening, GC-MS analysis and antioxidant activity of Ananas comosus using its peel sample.

Keywords*: Ananas comosus, GC-MS, Phytochemicals, Antioxidant, Neuraceuticals*

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INTRODUCTION

India is called the botanical garden of the world for its rich natural resources. Over 6,000 plants in India are used in traditional, folklore and herbal medicine. The Indian system of medicine has identified 1500 medicinal plants of which 500 are commonly used [1]. Phytochemistry or plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and is closely related to both. It is concerned with the enormous variety of organic substances that are elaborated with and accumulated by plants and deals with the chemical structures of these substances, their biosynthesis, turn over and metabolism, their natural distribution and their biological function [8].

Phytochemicals are the chemicals extracted from plants. These organic chemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlrophyll's etc. Secondary constituents are the remaining plant chemicals such as alkaloids (derived from aminoacids), terpenes (a group of lipids) and phenolics (derived from carbohydrates) [10]. Plant produces these chemicals to protect itself but recent research demonstrates that emphasizes the plant source of most of these protective, disease-preventing compounds. A true nutritional role for phytochemicals is becoming more probable every day as research uncovers more of their remarkable benefits [6].

Consumers are currently demanding less use of chemicals or minimally processed fruits and vegetables, so more attention had been paid to search for naturally occurring substances. This is particularly true for plant materials that act as alternative antioxidant sources. Medicinal herbs have been use in one form or another under indigenous systems of medicine [5]. The complete phytochemical investigations of medicinal plants of India should be carried out, because these secondary metabolites are responsible for medicinal activity of the plant.

Plant products have been part of phytomedicines since time immemorial. These can be derived from any part of the plant like bark, leaves, peel, flowers, seeds, etc [4,5]. i.e., any part of the plant may contain active components. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances. Such phytochemical screening of various plants is reported by many workers [15,2,3].

Medicinal plants have long history of use and their uses are wide spread in both developed and developing countries. In India, an Ayurvedic system evolved over 5,000 years ago and still in practice. The Rig Veda and Atharvana Veda have included more than 700 medicinal prescriptions [11] other system of medicine such as the Chinese, Unani and Siddha traditions have their roots in Ayurveda. All the medicinal systems mentioned above mostly based on the plants and plant products that are available in Indian region. These plants are widely used by all sections of the society whether directly as folk remedies or indirectly as pharmaceutical preparation of modern medicine [18]. *I*n recent times, focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems [13].

Fruit and vegetable peels in general are discarded in the majority of common fruits even when it is safe for consumption. Indeed, the peel is being recognized as one of the essential components of our diet as it contains many vital nutrients and non-nutrient compounds, which play an important role in well being. Eating the peels of fruits and vegetables could boost the nutritional intake of vitamins, combat cancer and increase the energy levels. Therefore, the present study was undertaken to distinguish the screening of Secondary metabolites from peels of *Ananas comosus* using various solvents.

MATERIAL AND METHODS

Preparation of Peel Powders

Good quality *Ananas comosus* was washed in a running tap water to remove the adhering dust and dirt. The peels were hand peeled using peeler and the peel portion was collected separately. Peels were shade dried and powdered. The powdered peel materials of 5gm weighed using an electronic balance and were crushed in 25 ml of sterile water, boiled at 50-60°C for 30 minutes on water bath and it was filtered through Whatman No.1 filter paper. Then filtrate was centrifuged at 2500 rpm for 15 minutes and filtrate was stored in sterile bottles at 5°C for further use [7].

Secondary metabolites Screening and Qualitative Analysis

Chemical tests were carried out on the petroleum ether, chloroform, ethanol and aqueous extracts using procedures to identify the phytochemicals as described by [16-17,7].

Test for Carbohydrates

To 2ml of extract, 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Purple colour formation indicated the presence of carbohydrates.

Test for Tannins

To 1ml of extract, 2ml of 5% ferric chloride was added. Formation of greenish black colour indicated the presence of tannins.

Test for Saponins

To 2ml of extract, 2ml of distilled water was added and shaken in a graduated cylinder for15 minutes lengthwise. Formation of 1cm layer of foam indicated the presence of saponins.

Test for Flavonoids

5ml of dilute ammonia solution was added to a portion of the aqueous filtrate of extract followed by addition of concentrated sulphuric acid. Appearance of yellow colouration indicated the presence of flavonoids.

Test for Alkaloids

To 2ml of extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagents were added. Presence of green colour indicated the presence of alkaloids.

Test for Anthocyanin and Betacyanin

To 2ml of extract, 1ml of 2N sodium hydroxide was added and heated for 5minutes at 100ºC.Formation of yellow colour indicated the presence of betacyanin.

Test for Quinones

To 1ml of extract, 1ml of concentrated sulphuric acid was added. Formation of red colour indicated the presence of quinones.

Test for Glycosides

To 2ml of extract, 3ml of chloroform and 10% ammonia solution was added. Pink colour formation indicated the presence of glycosides.

Test for Cardiac glycosides
To 0.5ml of extract, 2ml of glacial acetic acid and few drops of 5% ferric chloride were added. This was under layered with 1 ml of concentrated sulphuric acid. Brown ring formation at the interface indicated the presence of cardiac glycosides.
Test for Terpenoids
To 0.5ml of extract, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Red brown colour formation at the interface indicated the presence of terpenoids.
Test for Triterpenoids
To 1.5ml of extract, 1ml of Libemann–Buchard Reagent (acetic anhydride + concentrated sulphuric acid) was added. Formation of blue green colour indicated the presence of triterpenoids.
Test for Phenols
To 1ml of the extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of green colour indicated the presence of phenols.
Test for Coumarins
To 1 ml of extract, 1ml of 10% Sodium hydroxide was added. Formation of yellow colour indicated the presence of coumarins.
Test for Acids
1ml of extract was treated with sodium bicarbonate solution. Presence of effervescence indicated the presence of acids.
Structural Elucidation by Gas Chromatography–Mass Spectroscopy (GC-MS):
The importance of this technique is that it requires only microgram amounts of material, that it can provide an accurate molecular weight and that it may yield a complex fragmentation pattern which is often characteristic of (and any identity) that particular compound. Mass spectroscopy, in essence, consists of degrading trace amount of an organic compound and regarding the fragmentation pattern according to mass. The sample vapours diffuses in to the low pressure system of the mass spectroscopy where it is ionized with sufficient energy to cause fragmentation of the chemical bonds. The resulting positively charged ions are accelerated in the magnetic field which disperse and permits relative abundance measurements of ions of given mass to charge ratio. The resulting record of ion abundance versus mass constituents, the mass spectral graph, which thus, consists of a series of lines of various intensity at different mass units. In many cases, some if the parent compound will servive. The vapourization process will be recorded as a parent ion peak. Those compounds which are too involite to vapourizr in MS instrument are converted to trimethyl sily ethers, methyl ester or similar derivaties [8]
Mass spectroscopy is frequently used in conjuction with GLC amd the combimed operation provides a qualitive and quantitative identification of the many structurally complex components that may be present in a particular plant extract.
Plant Sample Extraction
10gm of powdered peel of *Ananas comosus* was soaked in 20ml of Absolute alcohol overnight and then filtered through a Whatman ® No. 41 filter paper (pore size 20 - 25 m) along with 2gm of sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with absolute alcohol. The filtrate is then concentrated by bubbling nitrogen gas into the solution and was concentrated to 1ml. The extract contains both polar and non polar phytocomponents.
GC-MS Analysis
GC/MS analysis of this extract was carried out using a Perkin Elmer GC Claurus 500 system and Gas Chromatograph interfaced to a Mass Spectrometer (GC/MS) equipped with an Elite-1 fused silica capillary column (30 m × 0.25 mm ID. ×1 μMdf composed of 100% Dimethyl poly siloxane). For GC/MS analysis, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min and an injection volume of 2 μl was employed (split ratio of 10:1). Injector temperature 250°C, Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative percentage amount of each constituent was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a TurboMass Ver5.2.0.
***In Vitro* antioxidant studies:**
The efficacy of the aqueous extract of *Ananas comsus* peelswas studied under *in vitro* conditions. The free radical scavenging activity of peels of the *Ananas comsus* against 1, 1-diphenyl-2-picrylhydrazyl (DPPH) [12] 2, 2'-azino-bis (3-ethylbenzthiazoline -6-sulfonic acid) (ABTS)(Re *et al.,* 1999), of the test sample was estimated according to the describe method.

RESULT AND DISCUSSION

The different extracts were subjected to qualitative tests for the identification of various phytochemical constituents as per the standard procedure. The result of preliminary qualitative phytochemical analysis on peels of different solvent extract of *Ananas comosus* were showed in (Table 1).The result showed the presence of Alkaloids in Aqueous extract of *Ananas comosus,* Sterols in Chloroform, Ethanol, Ethyl acetate and Aqueous extract of *Ananas comosus* Carbohydrate and Glycoside in Chloroform, Ethanol, and Aqueous. Tannin in Chloroform, Ethanol and Aqueous extract of *Ananas comosus.* Triterpenoids and Saponins in Ether, Chloroform, Ethanol, Ethyl acetate and Aqueous of *Ananas comosus.* Gums and Mucillages in Aqueous extract *and* Flavonoids in Chloroform, Ethanol, Ethyl acetate and Aqueous extract of *Ananas comosus.Fixed oils in* Aqueous extract of *Ananas comosus.*

The screening of Secondary metabolites from peels of *Ananas comosus* revealed the presence of various phytochemicals(Table 1) like alkaloids, glycosides, Flavonoids, tannins, steroids, saponins, terpenoids, steroids, anthocyanins, coumarins, and fatty acids. The results suggest that the phytochemical properties for *Ananas comosus peel* possess potential antioxidant and leads to the isolation of new and novel compounds. In order to promote Indian herbal drugs, there is an urgent need to evaluate the therapeutic potentials of the drugs as per WHO guidelines mentioned that 30% of the world wide sales of drugs is based on natural products. Traditional indigenous medicine is limited to small tribal and geographical areas called "little traditions" are excellent repositories of knowledge about medicinal properties of botanical sources importance.

Percentage yield of *Ananas comosus* peel ofvarious extracts:

Type of solvent % w/w

Petroleum ether-1.28,Chloroform-2.35,Ethyl acetate -3.64, Alcohol -6.48, Water-8.17

Table 1 Qualitative analysis of *Ananas comosus*fruit peels

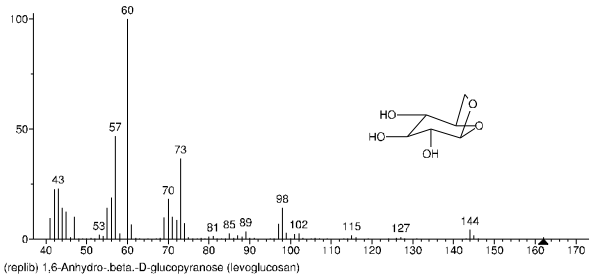
Constituents	Ether	Ethyl acetate	Chloroform	Ethanol	Aqueous
Alkaloids	-	-	-	+	++
Carbohydrates	-	-	-	-	+
Cumarines	-	-	+	-	+
Flavonoids	-	+	+	+	++
Fixed oils	+	-	+	-	+
Glycosides	-	-	+	++	++
Gums and reins	-	-	-	-	+
Mucilage	-	-	-	-	+
Saponins	-	-	+	-	+
Steroids and sterols	+	+	+	+	+
Tannins	-	-	+	+	++
Triterpenoids	+	+	+	-	++

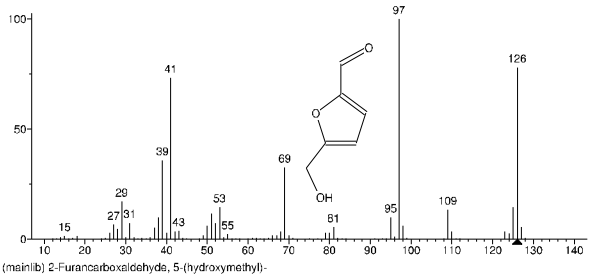
-= Negative; + = positive (low); ++ = positive (high)

PE- petroleum ether; CF- chloroform ; EA- ethyl acetate; ET- Ethanol; AQ- aqueous

GC_MS Study on phytoconstituents

The screening of Secondary metabolites from peels of *Ananas comosus* revealed the presence of various phytochemicals like alkaloids, glycosides, Flavonoids, tannins, steroids, saponins, terpenoids, steroids, anthocyanins, coumarins, and fatty acids. These are the compounds present in the ethanol extract of *Ananas comosus* peels. (Figure 1and 2)- 1,6-Anhydro-.beta.-D-glucopyranose (levoglucosan)$C_6H_{10}O_5$,,2-Furancarboxaldehyde, 5-(hydroxymethyl)$C_6H_6O_3$, 3-Trifluoroacetoxydodecane $C_{14}H_{25}F_3O_2$,3,4-Furandiol, tetrahydro-,trans-$C_4H_8O_3$,3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4 methylimidazoliumion $C_{24}H_{45}N_2O_3$, 3-Trifluoroacetoxydodecane -$C_{14}H_{25}F_3O_2$, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- $C_6H_8O_4$,6,11-Dimethyl-2,6,10-dodecatrien-1-ol- $C_{14}H_{24}O$,3,4-Furandiol, tetrahydro-, trans- $C_4H_8O_3$ 9-Octadecenal- $C_{18}H_{34}O$,9-Octadecenoic acid (Z)-, phenylmethyl ester-$C_{25}H_{40}O_2$, 9-Tetradecen-1-ol, acetate, (E)-$C_{16}H_{30}O_2$, alpha.-D-Glucopyranoside, O-.alpha.-D-glucopyranosyl-(1.fwdarw.3)-.beta.- D-fructofuranosyl- $C_{18}H_{32}O_1$,Cholestan-3-ol, 2-methylene-, (3.beta., 5.alpha.)-$C_{28}H_{48}O$, D-Glucose, 4-O-.alpha.-D-glucopyranosyl-**:** $C_{12}H_{22}O_{11}$,n-Hexadecanoic acid- $C_{16}H_{32}O_2$,Oleic Acid- $C_{18}H_{34}O_2$, Pentadecanoic acid, 2,6,10,14-tetramethyl-, methyl ester- $C_{20}H_{40}O_2$.


(replib) 1,6-Anhydro-.beta.-D-glucopyranose (levoglucosan)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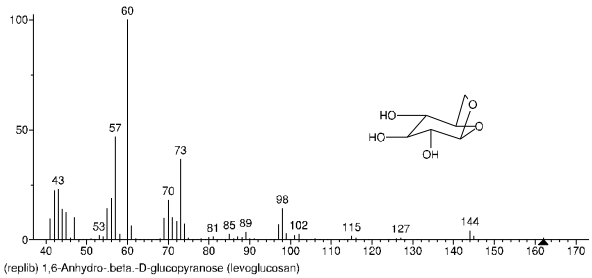
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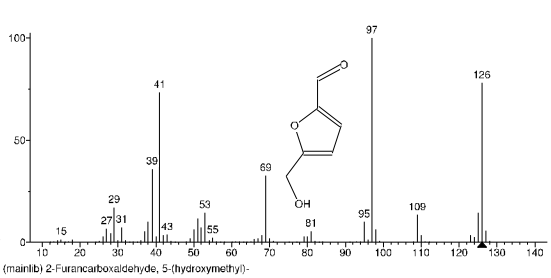
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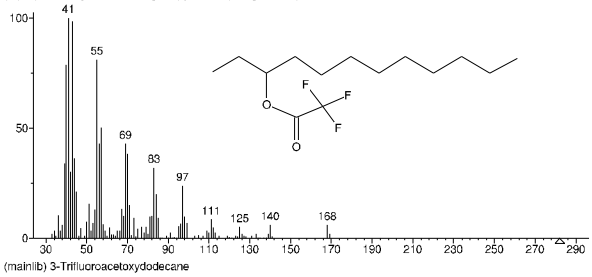
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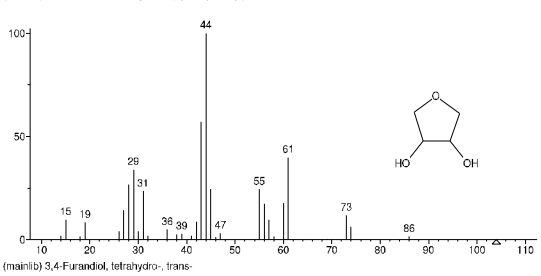
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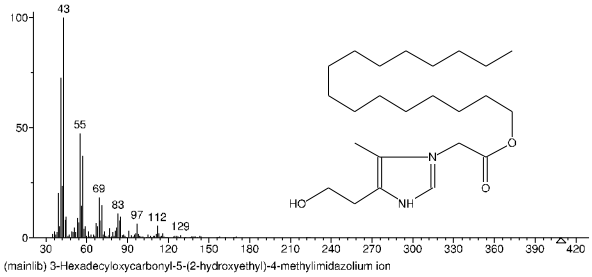
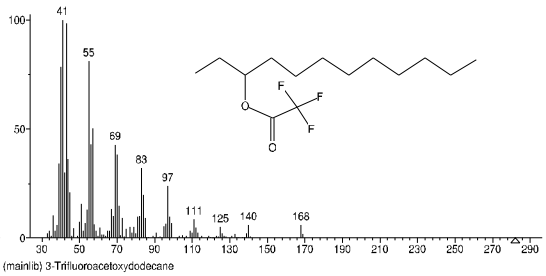

(mainlib) 3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion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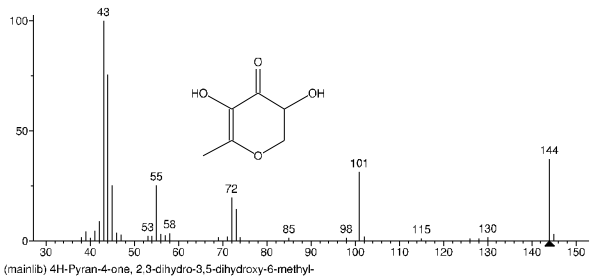
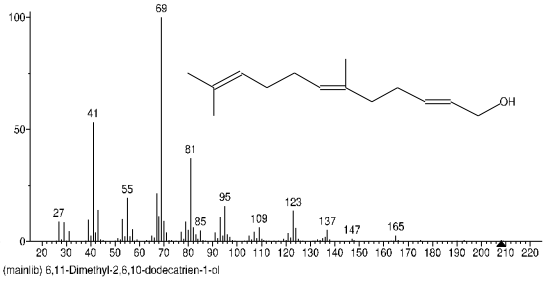

(mainlib) 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-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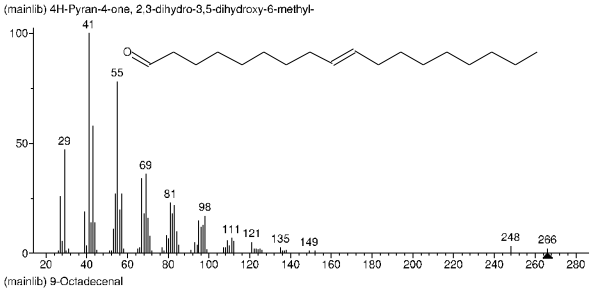
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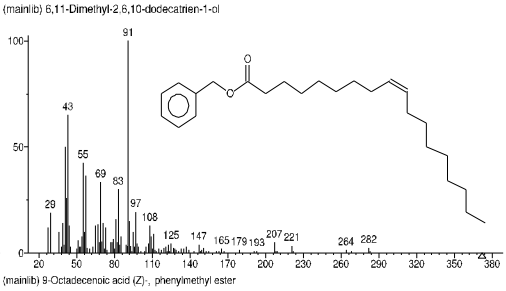
(mainlib) 9-Octadecenoic acid (Z)-, phenylmethyl ester

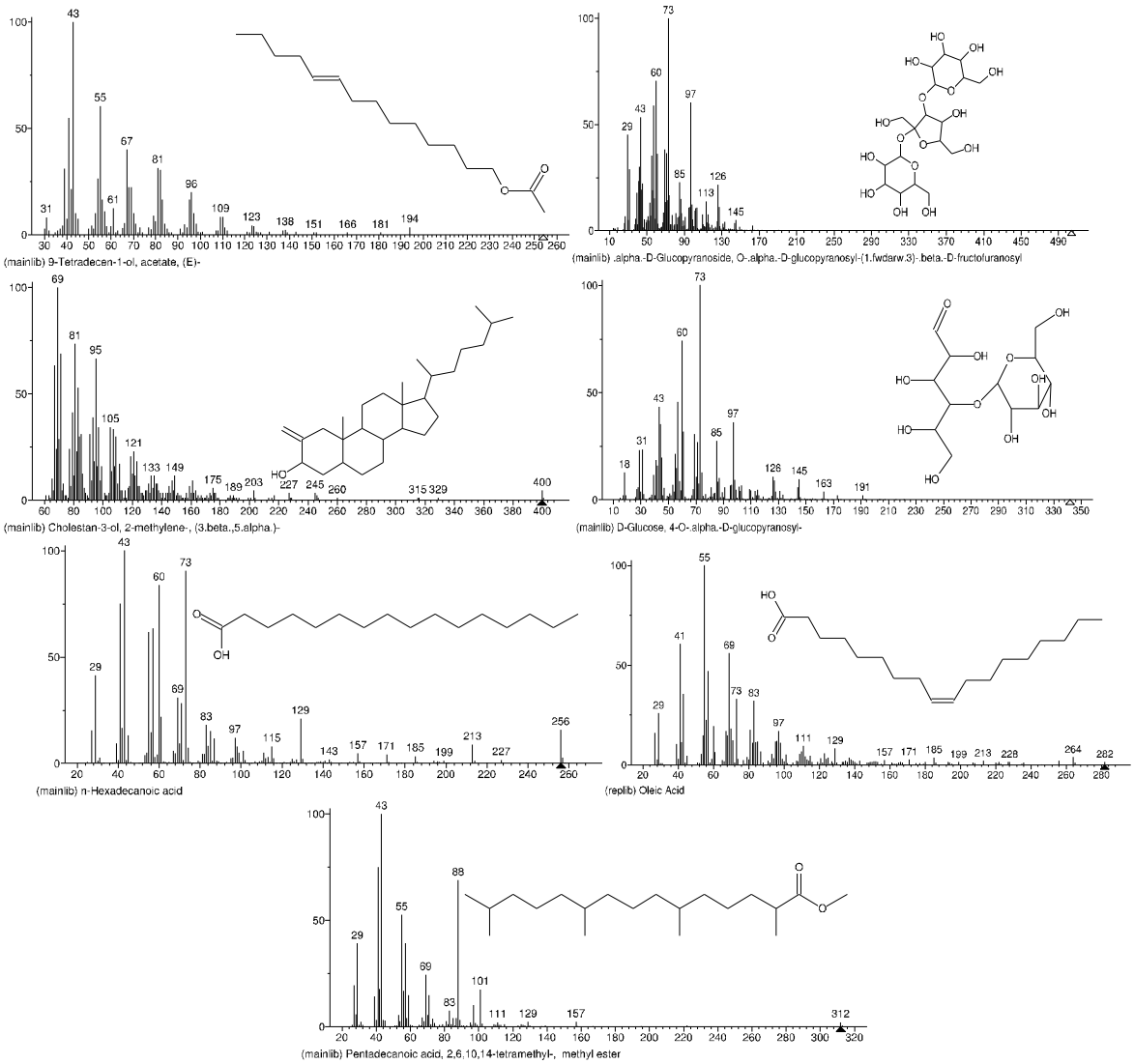

Fig. 1Compounds presents in ethanol extract of *Ananas comosus* peels

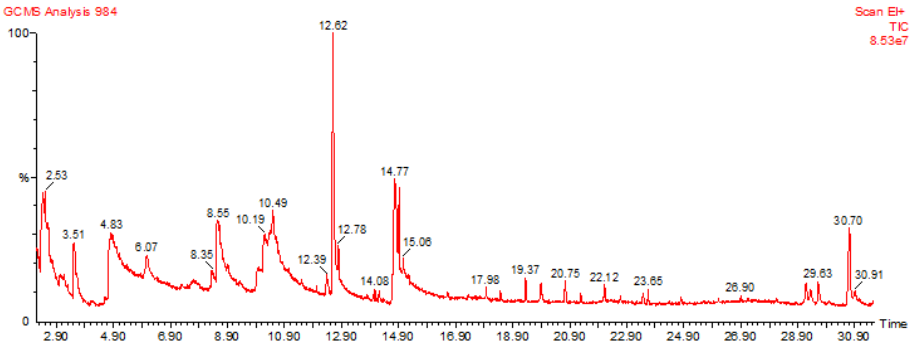

Fig. 2 Activity of Compounds identified in the *Ananas comosus* peel

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of the plant extract was presented in Figure 3. The percentage inhibition of DPPH by plant extract in different concentrations like 75, 150, 250, 500 µg/ml were observed as 35.11 ± 3.68,72.42 ± 7.19, 82.70 ± 1.55 and 90.24 ± 1.55 respectively whereas the percentage inhibition of ascorbic acid were found to be 43.41 ± 2.40, 65.50 ± 3.91, 85.64 ± 3.27 and 96.01 ± 4.43 respectively. The IC_{50} values for DPPH scavenging activity for aqueous extract of peels of *Ananas comosus* and ascorbic acid were 115µg/ml and 100µg/ml respectively. Values are the average of triplicate experiments and represented as mean ± standard deviation.

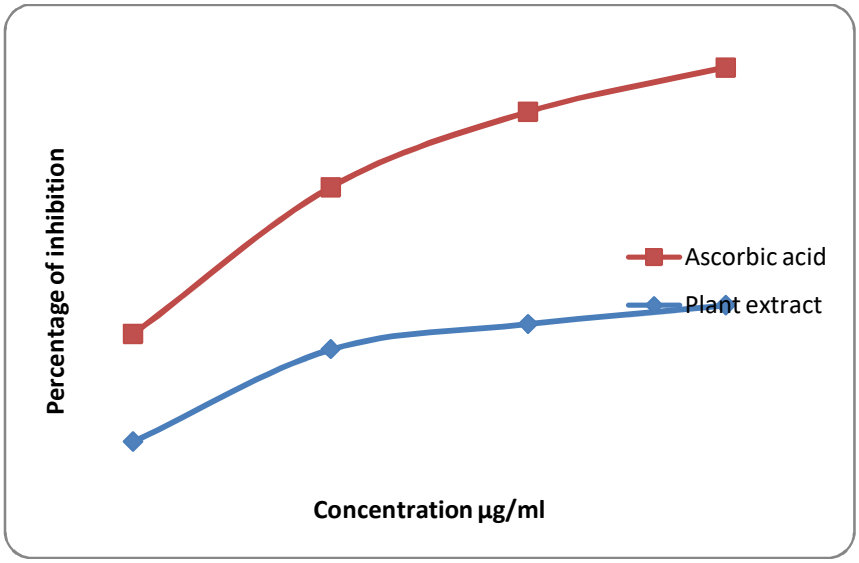

Fig. 3: Effect of Aqueous in *Ananas comosus* peel extract on DPPH scavenging activity

ABTS Radical Scavenging Activity

Figure 4 shows the percentage inhibition of ABTS by plant extract in different concentrations like 75, 150, 250, 500 µg/ml were observed as 34.44 ± 1.77, 52.62 ± 2.71, 75.51 ± 1.96 and 85.09 ± 2.31 respectively whereas the percentage inhibition of ascorbic acid were found to be 44.20 ± 2.02, 63.05 ± 2.86, 83.69 ± 2.20 and 96.52 ± 3.68 respectively. The IC_{50} values for ABTS scavenging activity for hydro aqueous extract of peels of *Ananas comosus* and ascorbic acid were 140µg/ml and 100µg/ml respectively. Values are the average of triplicate experiments and represented as mean ± standard deviation.

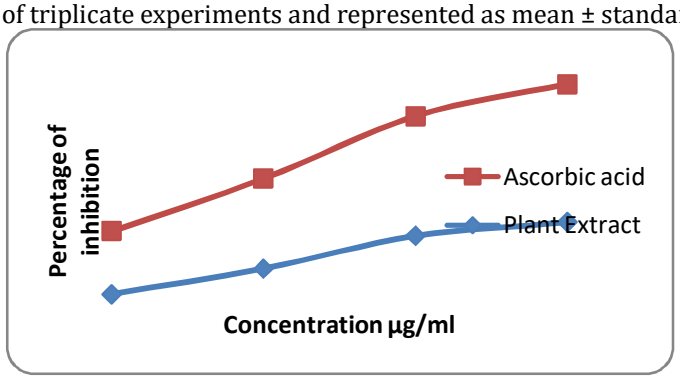

Fig. 4 Effect of Aqueous in *Ananas comosus* peel extract on ABTS radical scavenging activity

CONCLUSION

The medicinal plants appear to be rich in secondary metabolites, widely used in traditional medicine to combat and cure various ailments. The anti-inflammatory, antispasmodic, antianalgesic and antidiuretic can be attributed to their high steroids, tannins, terpenoids and saponins. Exploitation of these pharmacological properties involves further investigation of these active ingredients by implementation techniques of extraction, purification, separation, crystallization and identification.

The preliminary phytochemical screening of the leaves peel of *Ananas comosus peel* indicates the presence of secondary metabolities, having an essential role in medicine. The GC-MS screening revealed the presence of fatty acid, alkyl groups, and phenyl compounds.

The results obtained in the present study indicate that peel of *Ananas comosus* extracts exhibit significant free radical scavenging and antioxidant activity. The overall antioxidant activity might be attributed to its polyphenolic content andother phytochemical constituents. The findings of the present study suggest that peel of *Ananas comosus* could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases Overall, the present study provides some idea about phytochemical investigation on peel of *Ananas comosus.* This study paves the way for further attention/research to identify the active compounds responsible for the plant biological activity.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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