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# QUALITATIVE PHYTOCHEMICAL SCREENING, GC-MS ANALYSIS AND ANTIBACTERIAL ACTIVITY OF PALMYRA FRUIT PULP (*Borassus flabellifer L.*)

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## ABSTRACT

This study report the phytochemicals present in ethanolic extract of palmyra fruit pulp (PFP). Phytochemical screening of fruit pulp extract revealed the presence of saponins, flavonoids, phenolic compounds and glycosides. The extract was analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) and 10 different compounds were identified. Well diffusion method was employed to determine the effect of antibacterial potential against Gram positive bacteria i.e., *Staphylococcus aureus,* Gram negative bacteria i.e., *Escherichia coli*.

**KEY WORDS: Phytochemical screening, GC-MS analysis, Palmyra fruit pulp, Antibacterial activity**

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# INTRODUCTION

Traditional remedies have a long standing history in many locations in India and continue to provide useful and applicable tools for treating illness. For this reason, plant derived substances have recently become of great interest owing to their versatile applications[1]. Phytochemicals are compounds formed during the plant's normal metabolic processes[2] and are often referred to as secondary metabolites of which there are several classes, including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids[3]. These chemicals exert a significant physiological effect on the mammalian system[4].*Borassus flabelliferL*, belongs to family Arecaceae. In India it is called the tree with 800 uses[5]. The coconut like fruit is three-sided when young, becoming rounded or more or less oval, 12-15 cm wide, and capped at the base with overlapping sepals[6]. The fresh pulp is reportedly rich in vitamins A and C[7]. It has been used in traditional dishes and the sap, which was trapped from the flower part, has been used as a sweetener for diabetic patients[8]. The fresh sap is a good source of vitamin B-complex[6].According to vijayakumari et al [9] the PFP has good water and fat absorption properties. So PFP is used in bakery industry and in various food formulations.The Present investigation was carried out to determine the presence of various phytochemicals in ethanolic extract of palmyra fruit pulp by qualitative and GC-MS analysis. Antibacterial effect was also studied against clinical isolates of gram +ve and gram –ve organisms.

# MATERIALS AND METHODS

The collected fruits were sorted out in order to remove damaged ones and were separated on the basis of the state of ripeness, similarity in shape and size. The fruits were then washed, weighted, peeled and pulped. The pulp was stored at 4 $^0$C and some of the pulp was dried at 60$^0$C for 24 to 48 hrs. The dried pulp was finally milled using pulverizer to pass through 250 µm sieve. The samples were then packaged in polyethylene bag and kept in a refrigerator (4$^0$C) for further use[9]. Preparation of extractPalmyra fruit pulp powder was defatted with petroleum ether (60-80ºc) in a Soxhlet apparatus. The defatted powder material (marc) thus obtained was further extracted with ethanol (95% v/v). The solvent was removed by distillation under low pressure and evaporation. The resulting semisolid mass was vacuum dried by using rotary evaporator.

***Phytochemical analysis***

***Test for flavonoids***

To 1ml of the extract, a few drops of dilute sodium hydroxide was added. An intense yellow colour was produced in the plant extract, which become colourless on addition of a few drops of dilute acid indicates the presence of flavonoids[10].Test for saponins The extract (50mg) was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam showed the presence of saponins[11].

***Test for phenolic compounds***

The extract was dissolved in distilled water and to this few drops of 1% lead acetate were added a bulky white precipitate was formed, which indicates the presence of phenolic compounds[10].

***Test for Glycoside***

To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid is added, and observed for reddish brown colouration at the junction of two layers and the bluish green colour in the upper layer[12].

***Test for Tannins***

To 0.5 ml of extract solution, 1 ml of water and 1-2 drops of ferric chloride solution were added. Blue colour was observed for gallic tannins and green black for catecholic tannins[13].

***Test for alkaloids***

Extract was dissolved individually in dilute hydrochloric acid and solution was clarified by

filtration. Filtrate was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow color precipitate indicates the presence of alkaloids[14].

***Determination of antibacterial activity***

The clinical isolates, *E.coli* and *Staphylococcus aureus* were procured from local clinical laboratory, Vijayawada, A.P.., India. The agar well diffusion method was employed to determine the antimicrobial activities of the extract. A suspension of each sample of tested microorganism diluted prior to $10^{-1}$, $10^{-2}$ and $10^{-3}$ (1 ml of $10^8$ cells/ml) was spread on a solid agar medium in petri dishes (Nutrient agar). Wells of 6 mm size were made with sterile cork borer and test extracts were added. The agar plates were incubated at 37°C for 24 hrs. The diameter of zones of inhibition was measured in mm[15].

***Gas Chromatography–Mass Spectrometry (GC/MS) analysis***

GC-MS analysis of the extract was carried out by following the method of Kumaravel et al.[16]. GC/MS analysis was performed using a Perkin Elmer GC Claurus 500 system and Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Elite-1, fused silica capillary column (30 m x 0.25mmID x 0.25 µdf, composed of 95% Dimethyl poly siloxane). Helium gas 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 of compounds in sample obtained by electron ionization (EI) at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 min. The relative % amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo mass.

## RESULTS AND DISCUSSION

The qualitative analysis of the ethanolic extracts from the PFP sample showed the presence of phytochemical constituents such as saponins, flavonoids, glycosides and phenolic compounds. At the same time, Alkaloids and Tannins were absent (Table 1). Saponins are generally regarded as antinutrients but are also thought to be useful in human diet for controlling cholesterol. Its presence, therefore might suggest that the pulp powder has medicinal value. Flavonoids are a group of polyphenolic compounds which influence the radical scavenging, inhibition of hydrolytic and oxidative enzymes and also act as anti-inflammatory agent[17]. They also inhibit microbes which are resistant to antibiotics[18]. Phenolic compounds are of great importance as cellular support material because they form the integral part of cell wall structure by polymeric phenolics[19.] Bioactive polyphenols have attracted special attention because they can protect the human body from the oxidative stress which may cause many diseases, including cancer, cardiovascular problems and ageing[20]. Glycosides have been known to lower blood pressure[21].The results obtained in this study thus suggest that the identified phytochemical compounds may be the bioactive constituents.Antibacterial studies were carried out on human pathogenic bacteria, *E.coli* and *Staphylococcus aureus*. The extract showed significant antibacterial activity at high concentrations. As shown in table 2, 5mm was the highest zone of inhibition against *E.coli* (G-ve) at 150 mg concentration of extract. 4 mm was the highest zone of inhibition against *Staphylococcus aureus* (G+ve) at 150 mg concentration of extract. The extract showed comparable antibacterial activity with Ciprofloxacin (antibiotic).The study on ethanolic extract of the palmyra fruit pulp using GC- MS showed the presence of 10 major and minor peaks. The GC- MS chromatogram of the ten peaks of the compounds detected are shown in the figure 1. The major and minor compounds with their retention time (RT), molecular formulae, molecular weight (MW) and peak area (%) are presented in the Table 3.The GC

– MS analysis revealed that these phytochemicals are responsible for various pharmacological actions like antimicrobial activity. This study is only a preliminary study of the occurrence of certain properties of PFP.

**Table 1**
***Phytochemical screening of palmyra fruit pulp***

| Phytochemicals | Ethanolic extract |
|---|---|
| Flavanoids<br>Alkaline Reagent Test | Present |
| Saponins<br>Froth Test | Present |
| Phenolic compounds<br>Lead acetate test | Present |
| Glycosides | present |
| Alkaloids<br>Mayer's Test | Absent |
| Tannins<br>Ferric chloride test | Absent |

**Table 2**
***Antimicrobial activity of ethanolic extract of PFP on human pathogens.***

| S.No. | organism | Causative agent | Zone of inhibition | | | Antibiotic |
|---|---|---|---|---|---|---|
| 1. | *E.coli* | | 50 mg | 100 mg | 150 mg | Ciprofloxacin(50µg) |
| | | Urinary tract infections and gastrointestinal tract infections | 2 | 3.5 | 5 | 7 |
| 2. | *Staphylococcus aureus* | Skin and soft tissue infections | 3 | 3.5 | 4 | 8 |

| No. | RT | Name of the compound | Molecular formula | Molecular weight | Peak Area % |
|---|---|---|---|---|---|
| 1. | 5.44 | 2-furancarboxaldehyde, 5-(hydroxymethyl)- | $C_6H_6O_3$ | 126 | 59.16 |
| 2. | 1027 | a-D-Glucopyranoside, O-a-D-glucopyranosyl-(1 fwdarw.3)-a-D-Fructofuranosyl | $C_{18}H_{32}O_{16}$ | 504 | 4.73 |
| 3. | 11.43 | 5,9,13-Pentadecatrien-2-one, 6,10,14-trimethyl-(E.E)- | $C_{18}H_{30}O$ | 262 | 0.23 |
| 4. | 12.41 | n-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256 | 7.04 |
| 5. | 13.45 | Oleic Acid | $C_{18}H_{34}O_2$ | 282 | 0.25 |
| 6. | 14.56 | 9,12-Octadecadienoic acid (z,z)- | $C_{18}H_{32}O_2$ | 280 | 27.02 |
| 7. | 16.96 | Estr-1,3,5 (10)-trien-17a-ol | $C_{18}H_{24}O$ | 256 | 0.35 |
| 8. | 19.45 | Erucic acid | $C_{22}H_{42}O_2$ | 338 | 0.76 |
| 9. | 19.70 | 7-Methyl-Z-tetradeen-1-ol acetate | $C_{17}H_{32}O_2$ | 268 | 0.24 |
| 10 | 22.70 | Squalene | $C_{30}H_{50}$ | 410 | 0.22 |

**Table 3**
***Chemical components of the ethanolic extract of palmyra fruit pulp GC-MS analysis***

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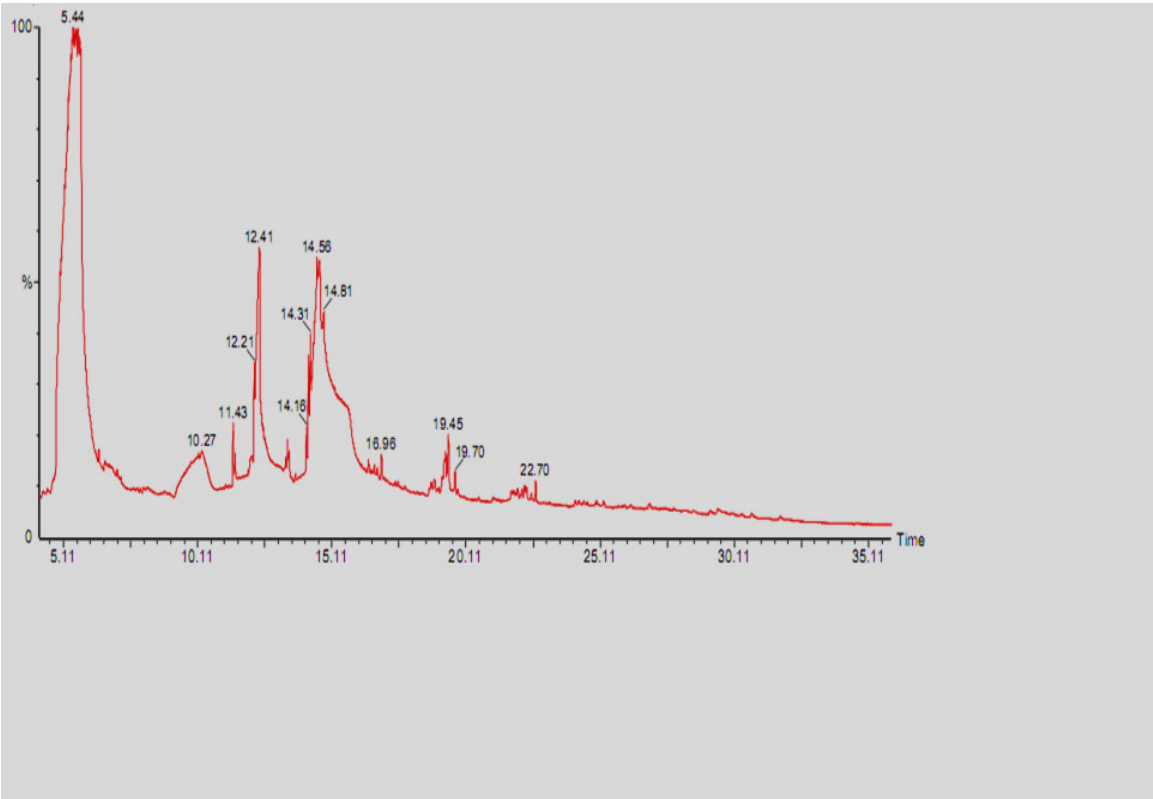


**Figure 1**
***GC-MS chromatogram of ethanolic extract of palmyra fruit pulp***

## CONCLUSION

The present study was focused in two areas of enquiry. The first area involved photochemical analysis and in the second part of the study, characterization of secondary metabolites was done using GC/MS analysis which revealed that these phytochemicals are responsible for various pharmacological actions. Therefore, the above results confirmed that the palmyra fruit pulp powder as a new valuable component for food and nutraceutical applications in the promotion of health.

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