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Phytochemical Studies Of Balanites Aegyptiaca (Yellow Natural Hingot) Seed Ethanol Extract And Antioxidant Activity.

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Abstract
The evergreen, thorny, spiny blooming tree Balanites aegyptiaca (L.) Del, often known as the "desert date," grows to a height of about 10m and is a fantastic medicinal source for curing diseases. It belongs to the Balanitaceea family, which is widely distributed in the arid regions of southern Asia and Africa. Saponins, flavonoids, alkaloids, lipids, proteins, carbohydrates, and organic acids are among its constituents. The present study, therefore, focusses on the phytochemicals that have antioxidant activity and are found in the Balanites aegyptiaca seed ethanol extract (BASEE). The presence of important phytochemicals such polyphenols, alkaloids, tannin, steroids, and triterpenoids was identified by the qualitative analysis of BASEE.
The findings from infrared spectrophotometry revealed the amide groups and heterogeneous functional groups (OH, C=C, =C-H). A chromatogram of BASEE using gas chromatography–mass spectrometry also showed the presence of several phytochemicals. BASEE showed DPPH scavenging activity of around 60%, with an IC50 value of 49µg/ml.

Keyword: *Phytochemicals, extract antioxidant.*

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I. Introduction

The evergreen dicotyledonous multibranched Savannah tree species known as *Balanites Aegyptiaca (L.)*, or Yellow Natural Hingot, is indigenous to dry and semi-arid regions of Africa, the Arabian Peninsula, and South Asia (1). For those living in rural areas, Balanites aegyptiaca is a forest species of socioeconomic relevance. This fruit, also known as "desert date," is highly nutritious and valuable to the local population (2). It is a thorny shrub or tree that grows up to 0 meters tall and is commonly found in dry, arid regions of South Asia and Africa. Many conditions, including jaundice, intestinal worm infections, wounds, malaria, epilepsy, insanity, yellow fever, syphilis, helminthiasis, coughing, and constipation, have historically been treated with it (3). Protein, fat, carbohydrates, alkaloids, saponins, flavonoids, and organic acids are all present (4). The fruit is a drupe that is hairy when green and turns yellowish and glabrous when it ripens. It is commonly known as desert date and lalobe in Arabic (5). It is composed of four layers: the woody shell called endocarp, the inner seed called kernel, the fleshy pulp called mesocarp, and the outer skin called epicarp. This plum-sized, somewhat five-grooved fruit is made up of four parts: the endocarp (49–54%), mesocarp (28–33%), epicarp (5%–9%), and kernel (8%–12%) (6). When ripe, the green, pubescent immature fruits become brownish-yellow and become glabrous, with a taste that is both sweet and tart. The fleshy pulp of the desert date fruit has a high sugar content (35%–42%), with reducing sugars making up 81.3%–91.1% of the total (7). Fruit kernels have a significant amount of nutritional oil (40%–51%), which is composed of 11 distinct fatty acids, mostly oleic and linoleic but also saturated (mostly palmitic and stearic) (8). Additionally, the fruit kernel has a high protein content (26.1%–34.3%) and a varied amount of polyphenols (47.8–117.5 mg/100 g), according to reports (9). Nonetheless, saponins, also known as balanitinis, continue to be the fruit of *B. aegyptiaca* with the highest known chemical composition; they make up 7.2% of the pulp and 6.7% of the kernel (10). The fruit of the desert date, together with its leaves, branches, and roots, have been shown to contain other bioactive substances like alkaloids, flavonoids, tannins, and vitamins (11). Alkanoids, tannins, and saponins were found in the stem bark of *B. aegyptiaca* using methanolic and acetone phytochemical screening; flavonoids, glycosides, and steroids were not present. used methanol and acetone to screen the stem bark of *B. aegyptiaca* and found terpens, tannins, volatile oils, and saponins; flavonoids, alkanoids, and glycosides were not present. The plant's screened leaves revealed the presence of flavonoids, phenols, tannins, amino acids, glycosides, carbs, and saponins, but not alkanoids. Numerous research have been carried out with the goal of assessing the nutritional value of different plant components (12). The researchers analysed the proximate composition of the different plant sections in order to determine the nutritive values. Therefore, an attempt was made to characterise the phytochemicals of the ethanol extract from *Balanites Aegyptiac* seeds in the current

study, and the findings are reported. Numerous lifestyle disorders are caused by unchecked oxidative stress. Medication used to address the aforementioned conditions may also have unfavourable effects by generating more ROS. Because plant extracts and isolated plant chemicals have the lowest side effects and the highest medicinal efficacy, they have gained interest recently. Despite the fact that seeds contain a number of phytochemicals with a wide range of medicinal uses, they have received little attention. The purpose of the study is to describe the phytoconstituents of desert date seed and its potential protective effect against oxidative stress.

II. Materials And Methods

Chemicals and Reagents

1,1-diphenyl-2-picrylhydrazyl (DPPH), alcoholic α-naphthol, NaOH, $CuSO_4$ solution. Alcoholic KOH, phenolphthalein, Hager's reagent (saturated picric acid solution), gelatin, sodium chloride sulphuric acid, 10% of ammonia were purchased from Sigma Chemical Company (St. Louis, USA).

Preparation of *Balanites Aegyptiaca* seed ethanol extracts (BASEE)

The *Balanites Aegyptiaca* fruits were purchased from the local market in Bangalore, washed thoroughly, and air-dried for 24 h. The epicarp, mesocarp and endocarp were mechanically separated. The kernel part of the fruit was dried, 10g dried seed powder were poured into 200ml conical flask containing 50ml of ethanol. The mixtures were gently stirred then tightly covered with a cork stopper and left for 24h to macerate the mixture was filtered into another conical flask through a funnel choked with non-absorbent cotton. The resultant filtrate was completely dissolved in water before being utilized for all tests.

Qualitative phytochemical analysis

Harbone et al. (13) evaluated the acquired *Balanites Aegyptiaca* seed ethanol extracts (BASEE) for the presence of the preliminary phytochemical analysis using conventional procedures.

Test for carbohydrates

In a test tube, BASEE (1ml) was treated with two drops of an alcoholic α-naphthol solution. The presence of carbohydrates was shown by the formation of a violet ring at the junction.

Test for lipids

After treating BASEE (1ml) with 10% NaOH solution, two drops of 0.1% $CuSO_4$ solution were added. The presence of proteins was indicated by the formation of the violet pink colour.

Test for alkaloids

BASEE was dissolved in the chloroform, which then evaporated, acidifying the residue and releasing a little amount of Wagner's reagent (potassium iodide containing iodine). Precipitation turned orange.

Test for tannins

In the test tube, 2ml of BASEE was poured, and then drops of ferric chloride solution were added. There was a blue-black precipitation visible.

Test for steroids

1ml of BASEE was combined with a few drops of boiling, cooled acidic anhydride. Subsequently, strong sulphuric acid was added to the test tube walls. The presence of steroids was revealed by the creation of a brown ring at the intersection of two layers.

Test for flavonoids

A few drops of neutral ferric chloride solution were added to 1ml of BASEE. The phenolic nucleus caused the color to turn green.

Test for phenols

After treating BASEE (1ml) with 5% ferric chloride, the presence of phenol was confirmed by the creation of a deep blue black blue.

Glycosides test

BASEE (1ml) was treated with 1ml of glacial acetic acid and adds few drops of ferric chloride solution then slowly sulphuric acid was introduced through the walls of the test tube. There was a reddish-brown ring visible at the junction of the liquids.

Test for triterpenoids

A few drops of sulphuric acid were added to 1ml of BASEE, agitated and left to stand. Diminished layer became yellow.

Fourier Transform Infrared Spectrophotometry (FTIR)

The infrared spectrum was recorded using an FTIR spectrophotometer (Thermo Nicolet iS50-Thermo Fisher Scientifc, USA). The method of Kareru et al. (14) was followed. In brief, BASEE (2 µg) was mixed with potassium bromide salt and compressed into a thin pellet. The infrared spectrum was recorded as a KBr pellet and scanned in the range from 400 to 4000 cm−1 with a resolution of 4 $cm^{-1.}$

Gas Chromatography–Mass Spectrometry Analysis (GC- MS)

GC–MS analysis was carried out using an Agilent 6890 gas chromatograph and a 5975B mass spectrometer (USA) in trace ion detection mode with a PTV injector. On a capillary column, the chromatographic separation was carried out (30×0.25 mm IDX1EM df, composed of 100% dimethylpolysiloxane). At 280 °C, the extract (2 µl) was injected by an empty bafed liner in the split mode (1:50). The National Institute of Standards and Technology (NIST05) database, which has more than 62,000 patterns, was used to interpret the GC–MS spectra.

Determination of the antioxidant activity by the DPPH assay

The Okoh et al. technique (15) was used to assess the DPPH radical scavenging activity. Using 95% ethanol, DPPH (0.04 mg/100 mL) radical solution was created. BASEE (10–50µg) was dissolved in water and combined with 150µL of DPPH radical solution. The final volume was increased to 600µL by adding 95% ethanol, and it was then allowed to sit at room temperature for 30 minutes in the dark. Using a spectrophotometer (Thermo Scientific Biomate 6, USA), the absorbance was measured at 517nm. Ascorbic acid served as a positive control and ethanol as a blank. The following formula was used to compute the scavenging activity.

$$DPPH\ scavenging\ activity\ (\%) = \frac{(Absorbance\ of\ control - Absorbance\ of\ the\ sample)}{(Absorbance\ of\ control)} \times 100$$

BASEE's antioxidant activity was expressed in terms of IC50. The concentration of the extract (µg/ml) needed to suppress the production of DPPH radical by 50% was established as the IC50 value.

III. Result And Discussion

The qualitative analysis of BASEE revealed the presence of key phytochemicals such as polyphenols, alkaloids, tannin, steroids and triterpenoids (Table1). BASEE was subjected to FTIR analysis; an absorption band at 3219cm−1 denoted the O-H hydroxyl group, alkylene stretching =C-H (cis) bending were observed at 1566cm−1 and 1406 cm−1, In addition there was a amide or amine groups, C-O stretching at the peak 1253 cm−1 and 1019 cm−1 respectively (Fig 01). Furthermore, the GC–MS data of BASEE revealed sharp peaks at the retention time of 10.63min (2-Stearoylglycerol $C_{21}H_{42}O_4$),13,53min (2-Formyl-9-ribofuranosyl hypoxanthine ($C_{11}H_{12}N_{O46}$)),13.92min(Benzene,1-(1,5-dimethyl-4-hexenyl)-4-meth($C_{15}H_{22}$)),15.66min(1,6,10-Dodecatrien-3-ol,3,7,11-Trimethyl-($C_{15}H_{26}O$)), 19.71min(Spiro[4.5]dec-6-en-8-one, 1,7-dimethyl ($C_{15}H_{24}O$)), 21.42min (2,2í-(m-Phenylene)dithiophene), 23.25min (Methyl 3-(1-formyl-3,4-methylenedioxy) benzoate), 25.17min (3-Decanone ($C_{17}H_{26}O_3$)), and 27.17min (Gingerol ($C_{17}H_{26}O_4$). The examined molecular mass was found to be 77.11, 105.10, 112.10, 122.10, 122.08, 129.12, 166.09, 207.12 kD respectively (Fig 02). Using the DPPH free radical scavenging method, BASEE antioxidant capacity was assessed. It demonstrated 60% radical scavenging activity, with an average IC_{50} value of 45.29µg. The ethanol extract from *Balanites Aegyptiaca* seeds contained alkaloids, tannins, steroids, polyphenols, and triterpenoids, according to both qualitative and quantitative investigation. The presence of aldehyde, alkyne, and hydroxyl molecules that may be in charge of scavenging the free radicals produced during oxidative stress was confirmed by the results from FTIR and GC-MS. high antioxidant qualities are exhibited by secondary metabolites like alkaloids, polyphenols, tannins, flavonoids and triterpenoids, which have been described by multiple plant sources (16). BASEE demonstrated high antioxidant characteristics by scavenging the DPPH radical. DPPH has the ability to take in an electron or hydrogen and change it into a stable diamagnetic molecule at room temperature. The drop in the optical density of the DPPH free radical indicates its reducing property. Through hydrogen donation, free radicals are quenched as a result of the interaction between antioxidant molecules and free radicals. It appears as a visual shift in color from purple to yellow. According to the antioxidant principle (17), the BASEE may react with hydrogen donors to convert free radicals to the equivalent hydrazine.

IV. Conclusion

The free radicals linked to phytochemicals such triterpenoids, alkaloids, glycosides, tannins, and steroids were effectively neutralised by BASEE.

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Figure Legend

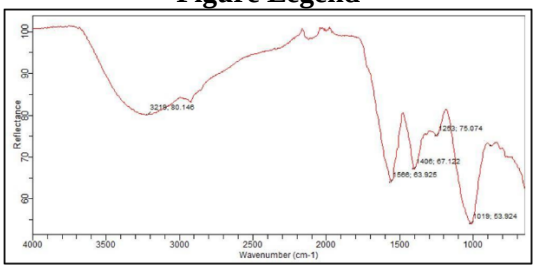

Fig 01: FTIR spectrum of BASEE: IRspectrum of BASEE was done using Agilent FT-IR-4100 spectrophotometer at the spectral range of 650– 4000 cm^{-1} in AT disk.

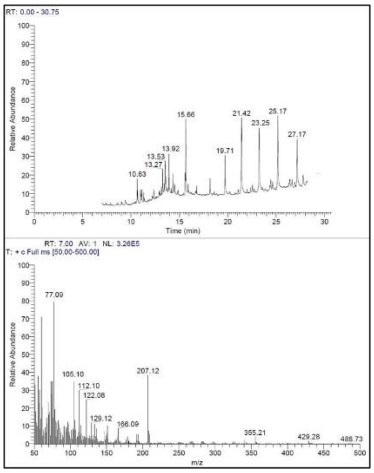

Fig 02: GC–MS chromatogram of BASEE. BASEE (5µl) was analyzed in GC–MS with a single quadrupole mass spectrometer in the electron-capture negative-ion chemical ionization (ECNICI) mode with a capillary column (30×0.25 mm). Helium was used as carrier gas at the fow rate of 1 ml/min with a gradient temperature system and the injection volume of 0.5 El (split ratio of 10:1)

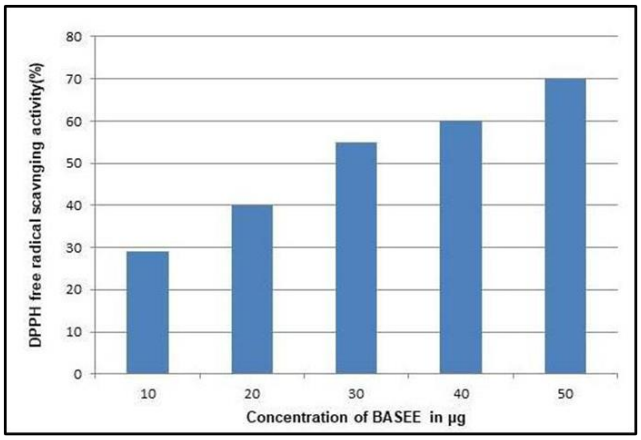

Fig 03: DPPH scavenging activity of BASEE. Antioxidant activity of BASEE measured by DPPH method.

Table 1
Phytochemical analysis of BASEE

Preliminary test	Results
Carbohydrates	–
Proteins	–
Lipids	–
Alkaloids	+
Tannins	+
Steroids	+
Flavonoids	–
Phenols	+
Glycosides	–
Triterpenoids	+