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EXTRACTION, PHYTOCHEMICAL SCREENING AND ANTIPYRETIC ACTIVITY OF *BALANITES AEGYPTIACA*

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ABSTRACT

This study aimed to explore the potential antipyretic activity of *Balanites aegyptiaca* through extraction, phytochemical screening, and evaluation of its biological properties. The methanolic extract of *Balanites aegyptiaca* exhibited a yield of 7.6% w/w and was subjected to phytochemical analysis, revealing the presence of carbohydrates, saponins, phenols, and alkaloids, while proteins, diterpenes, glycosides, flavonoids, tannins, and specific alkaloids were absent. Quantitative analysis showed the extract contained 0.35 mg/100 mg of total phenols and 0.72 mg/100 mg of total flavonoids. To assess its antipyretic potential, the extract was tested using a yeast-induced pyrexia model in rats. The results demonstrated that administration of the extract significantly reduced rectal temperatures compared to the control group over a 3-hour period. Group III, treated with the extract, showed notable temperature reduction, suggesting effective antipyretic activity. These findings highlight *Balanites aegyptiaca* as a potential source of antipyretic agents, likely mediated through its antioxidant constituents. Further research is warranted to elucidate the underlying mechanisms and to explore clinical applications for managing fever-related conditions.

Keywords: *Balanites aegyptiaca,* antipyretic potential, methanolic extract, phytochemical analysis

INTRODUCTION

Balanites aegyptiaca, commonly known as desert date or soapberry tree, is a versatile plant species native to arid regions of Africa and the Middle East. It holds a significant position in traditional medicine due to its rich reservoir of bioactive compounds found in various parts of the plant, including the bark, leaves, fruits, and roots. The plant's medicinal properties have been attributed to its diverse phytochemical composition, encompassing alkaloids, saponins, flavonoids, tannins, glycosides, and phenolic compounds (Elleuch *et al.,* 2008; Abdel-Sattar *et al.,* 2018).

Phytochemical investigations of *Balanites aegyptiaca* have revealed its potential therapeutic benefits, including antioxidant, anti-inflammatory, antimicrobial, and antipyretic activities. These properties have sparked interest in exploring the plant's pharmacological potential and identifying novel bioactive agents for various medical applications. Extraction methods using solvents such as ethanol, methanol, and water have been employed to isolate and concentrate these bioactive compounds, facilitating their characterization and evaluation (Hammouda *et al.,* 2013; El-Fiky *et al.,* 2004).

Among its pharmacological activities, the antipyretic effect of *Balanites aegyptiaca* is of particular interest.

Fever, a common symptom of infectious and inflammatory conditions, is characterized by elevated body temperature. Experimental studies have demonstrated the plant's ability to reduce fever, suggesting its potential as a natural antipyretic agent. This antipyretic activity has been attributed to specific phytochemicals present in *Balanites aegyptiaca* extracts, although further research is needed to elucidate the underlying mechanisms (El-Fiky *et al.,* 2004).

This study aims to explore the extraction methods, conduct comprehensive phytochemical screening, and evaluate the antipyretic activity of Balanites aegyptiaca extracts. The extraction process will involve optimizing protocols using different solvents to obtain bioactive constituents efficiently from different plant parts. Phytochemical screening will focus on identifying and quantifying alkaloids, saponins, flavonoids, tannins, glycosides, and phenolic compounds using established qualitative and quantitative methods. Subsequently, the antipyretic activity will be assessed using experimental models to evaluate the ability of the extracts to reduce fever induced by pyretic agents. Statistical analysis will be employed to interpret the data and draw conclusions regarding the efficacy of *Balanites aegyptiaca* as a potential source of antipyretic agents. The findings of this study are expected to contribute valuable insights into the medicinal properties of *Balanites aegyptiaca*, potentially paving the way for further research and development in pharmacological applications.

MATERIALS AND METHODS

Collection of plant materials

Plants can be collected from either wild woods or herbariums. However, there is a risk of erroneously recognized plants in the case of wild plants. They have the advantage of not containing any pesticides or herbicides. They are treated as quickly as possible after collection to avoid secondary metabolites from deteriorating. The fruits of selected plant namely *Balanites aegyptiaca* were identified and collected from local area of Bhopal on the basis of geographical availability.

Figure 1: Fruits of *Balanites aegyptiaca*

Extraction using microwave assisted extraction technique

Extraction, defined as the separation of medicinally active portions of plant tissues from the inactive components using solvents, involves the diffusion of the solvent into the plant material (marc) and solubilizing compounds with similar polarity. In this study, the shade-dried fruits of *Balanites aegyptiaca* were coarsely powdered and subjected to extraction. Methanolic solvent was utilized for this purpose. Subsequently, 40 grams of this powder were subjected to extraction via the microwave-assisted extraction technique. The resultant extract was then centrifuged at 7000 rpm for 10 minutes.

The supernatant was carefully collected into Petri plates, and the solvent was allowed to evaporate at room temperature. Once the solvent had completely evaporated, the resultant powder was scraped from the Petri plates and stored for further analysis (Mukherjee, 2007).

Determination of percentage yield

Percentage yield measures the effectiveness of the entire extraction process. It shows how much product a researcher has obtained after running the procedures against how much is actually obtained. A higher % yield means the researcher obtained a greater amount of product after extraction. The % yield was calculated by using formula:

% Yield = [(weight of dried extract) / (weight of dried plant sample)] x 100

Phytochemical screening

Plants generate compounds known as phytochemicals. These are created by the primary and secondary metabolisms of the plant. These phytochemicals are necessary for plants to survive or to fend off other plants, animals, insects, microbial pests, and pathogens. They also protect plants from illness and damage induced by environmental threats such as pollution, UV, stress, and drought. They have been employed as traditional medicine and as poisons since ancient times (Kokate, 1994; Harborne, 1998).

Test for alkaloids

1. Hager's test: to a few ml of filtrate, 2 drops picric acid was added formation of yellow precipitate shows a positive result for alkaloids.

2. Wagner's test (iodine **–** potassium iodine reagent): To about an ml of extract few drops of Wagner's reagent were added. Reddish – brown precipitate indicates presence of alkaloids.

Test for phenol

A) FC reagent test: To 5ml of extract 2ml of Folin Ciocalteu reagent is added. Appearance of blue green colour indicates the presence of phenol.

B) Ferric chloride test: To 5 ml of extract few drops of feeric chloride solution was added and mixed gently. The production of blueish black colour solution indicate presence of phenols.

Test for flavonoids

A) Alkaline reagent test: To 5ml of extract 2ml of NaOH was added by which solution turns yellow colour, further dilute HCl (0.1 N) was added the solution becomes colourless which indicates the presence of phenol.

B) Lead acetate test: To 5 ml of extract few drops of lead acetate solution was added and mixed gently. The production of bulky white precipitate is positive for flavonoid.

Test for carbohydrate

A) Benedict's test: About 0.5 ml of the filtrate was taken to which 0.5 ml of Benedict's reagent is added. This mixture was heated for about 2 minutes in a boiling water bath. The appearance of red precipitate indicates the presence of sugars.

B) Fehling test: About 0.5 ml of the filtrate was taken to which 0.5 ml of each Fehling A & Fehlinng B solution was added. This mixture was heated for about 2 minutes in a boiling water bath. The appearance of red precipitate indicates the presence of sugars.

Detection of proteins and amino acids

Xanthoproteic Test: The extract was treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins and amino acids.

Detection of diterpenes

Copper acetate Test: Extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Detection of glycosides

H_2SO_4 test: Extract was treated with dil. H_2SO_4, formation of red color solution indicate the presence of glycosides.

Detection of saponins

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Quantitative estimation of phenols and flavonoids

Natural antioxidants such as phenols, flavonoids and tannins are increasingly attracting because they are natural disease preventing, health promoting and anti-ageing substances. These conditions can cause DNA and protein damage, lipid peroxidation, cancer, ageing and inflammatory activity. Phenolics are an important class of secondary plant metabolites possessing an impressive array of pharmacological activity. Flavonoids have potent antioxidant qualities that help to protect the body from harmful poisons and counteract oxidative stress. Including foods high in flavonoids in your diet is a terrific approach to improve your general health and perhaps lessen your risk of diabetes, cancer, cardiovascular disease, and neurodegenerative disease.

Estimation of total phenolic content

The total phenolic content of dry extract was performed with folin-ciocaltaeu assay. 2 ml of sample (1 mg/ml) was mixed with 1 ml of folin ciocalteu's phenol reagent and 1 ml of (7.5 g/l) sodium carbonate solution was added and mixed thoroughly (Parkhe and Bharti, 2019). The mixture was kept in the dark for 10 minutes at room temperature, after which the absorbance was read at 765 nm. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as 100 milligrams of Gallic acid equivalents (GAE)/100mg of dried sample.

Estimation of total flavonoids content

Preparation of standard solution 10mg quercetin was weighed and made up to 10ml with methanol in a 10ml volumetric flask. From the above solution (1mg/ml), 1ml was pippeted out and made up to 10ml with methanol to get 100µg/ml Quercetin standard solution (stock solution). From the stock solution, solutions of concentration 5, 10, 15, 20 and 25 µg/ml were prepared (Parkhe and Bharti, 2019). 3 ml of each standard and test was mixed with 1 ml of 2% Aluminium chloride solution. The solutions were mixed well and the absorbance was measured against the blank at 420nm using UV-Visible spectrophotometer. A standard graph was plotted using various concentrations of Quercetin and their corresponding absorbance.

In vivo* anti-pyretic activity of *Balanites aegyptiaca

Animals

Wistar rats (150–200 g, 10-12 weeks old) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments.

The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Chemicals

All the chemicals used were of analytical grades, obtained from commercial suppliers.

Acute oral toxicity study

Toxicity studies were carried out by OECD guidelines, acute oral toxicity study of *Balanites aegyptiaca* fruits. Acute toxicity study was performed based on OECD guideline no. 423. The rats were assessed for signs of toxicity throughout the next 14 days. *Balanites aegyptiaca* fruits were given orally by the safe dose. Clinical symptoms like behavioural alterations, changes in the eyes, body weight, skin and fur were noted (Gilani *et al.,* 2022; Kazmi *et al.,* 2023).

Experiment design

Body weights of the animals were recorded and they were randomly divided into 5 groups of 6 animals each as follows:

Group I served as normal

Group II served as control- animals were treated with yeast via subcutaneous injection (10ml/kg).

Group III animals were administered with yeast (10 ml/kg) and the standard drug paracetamol (150mg/kg b.w.), orally

Group IV animals were administered with yeast (10ml/kg,) and with *Balanites aegyptiaca fruits* (100mg/kg b.w.), orally

Group V animals were administered with yeast (10ml/kg,) and with *Balanites aegyptiaca fruits* (200mg/kg b.w.), orally.

Yeast induced pyrexia

Pyrexia was induced by the subcutaneous injection of 20% w/v of brewer's yeast (10ml/kg) in distilled water. The basal rectal temperature was measured before the yeast injection, by inserting a digital clinical thermometer to a depth of 2 cm into the rectum. The rise in rectal temperature was recorded 18 h after yeast injection. Paracetamol 150mg/kg body weight was used as the standard antipyretic drug. Rectal temperature of animals was noted at regular intervals following the respective treatments. The temperature was measured at 1^{st}, 2^{nd} , and 3^{rd} hour after drug administration (Al-Saaedi, 2021).

Statistical analysis

The values were expressed as mean ± SEM (n=6). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Tukey's test and $P<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

The study on *Balanites aegyptiaca* explored its potential as an antipyretic agent through rigorous extraction, phytochemical screening, and evaluation of its biological activities. The

methanolic extract of *Balanites aegyptiaca* demonstrated a moderate yield of 7.6% w/w, indicating efficient extraction of bioactive compounds from the plant material. Phytochemical screening revealed the presence of carbohydrates, saponins, phenols, and alkaloids in the extract, as indicated by positive tests such as Benedict's, Foam, FC reagent, and Hager's tests, respectively. Conversely, tests for proteins, diterpenes, glycosides, flavonoids, tannins, and specific alkaloids were negative, suggesting their absence in the extract.

Quantitative analysis further characterized the extract, revealing a total phenol content of 0.35 mg/100 mg and a total flavonoid content of 0.72 mg/100 mg. These findings underscored the presence of antioxidant constituents in *Balanites aegyptiaca*, which are known to contribute to its pharmacological activities, including antipyretic effects.

The antipyretic activity of *Balanites aegyptiaca* was evaluated using a yeast-induced pyrexia model in rats. The results demonstrated significant temperature reduction in groups treated with the extract compared to the control group (Group II). Specifically, Group III (treated with the extract) showed a consistent decrease in rectal temperature over 3 hours, indicating its potential to alleviate fever induced by yeast injection. This observation aligns with the traditional use of *Balanites aegyptiaca* in treating febrile conditions.

The presence of phenolic compounds and their antioxidant properties likely contribute to the observed antipyretic effects by reducing oxidative stress and inflammation associated with fever. The findings support further exploration of *Balanites aegyptiaca* as a natural source of antipyretic agents and underscore its potential therapeutic value in managing fever-related conditions.

Table 1: % Yield of crude extract

Extract	Colour	Consistency	Yield (% w/w)
Methanolic	Brown	Solid	7.6%

Table 2: Preliminary qualitative phytochemical tests for *Balanites aegyptiaca* extract

Phytoconstituents	Phytochemical tests	*Balanites aegyptiaca* extract
Carbohydrates	Benedict's test	+ve
	Fehling test	+ve
Proteins and amino acids	Xanthoproteic Test	-ve
Diterpenes	Copper acetate Test	-ve
Glycosides	H_2SO_4 test	-ve
Saponins	Foam Test	+ve
Flavonoids	Alkaline reagent test	-ve

	Lead acetate test	+ve
Tannins	Gelatin Test	-ve
Phenol	FC reagent test	+ve
	Ferric chloride test	+ve
Alkaloids	Hager's test	+ve
	Wagner's test	-ve
'+ve' = Present; '-ve' = Absent		

Table 3: Total bioactive constituents content of *Balanites aegyptiaca*

S. No.	Extract	Total phenol	Total flavonoid
		mg/ 100mg	
1	Methanolic extract	0.35	0.72

Table 4: Antipyretic activity of *Balanites aegyptiaca* fruits against yeast induced pyrexia in rats

Rectal Temperature in °C after 18hrs of Yeast Injection				
Group	0 hr (±SEM)	1 hr (±SEM)	2 hr (±SEM)	3 hr (±SEM)
Group I	39.8 (0.9)	39.4 (0.8)	39.6 (0.5)	39.1 (0.8)
Group II	43.6 (0.15)	42.5 (0.15)	41.9 (0.11)	42.0 (0.12)
Group III	41.9 (0.13)	40.7 (0.13)	40.1 (0.11)	39.3 (0.10)
Group IV	42.1 (0.13)	41.3 (0.13)	40.7 (0.10)	40.2 (0.10)
Group V	42.5 (0.13)	41.3 (0.13)	40.2 (0.11)	39.6 (0.10)

Values expressed as mean ± SEM (n=6) $^{*}P<0.05$as compared to arthritis Control

CONCLUSION

In conclusion, the study provides valuable insights into the phytochemical composition and pharmacological activities of Balanites aegyptiaca, particularly its promising antipyretic properties. Future research can delve deeper into elucidating the mechanisms of action and conducting clinical trials to validate its traditional uses in therapeutic applications.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

REFERENCES

- Abdel-Sattar, E., Harraz, F.M., Ghareeb, D.A. et al (2018) Chemical composition, antioxidant and antitumor potentials of Annona muricata L. peel extracts. *Journal of Applied Pharmaceutical Science*, 8, 1–9.
- El-Fiky, F.K., Abou-Karam, M., Afifi, A. et al (2004) Phytochemical studies of Balanites aegyptiaca. *Fitoterapia*, 75, 508–511.
- Elleuch, M., Besbes, S., Roiseux, O., Blecker, C., Deroanne, C., Drira, N.E. & Attia, H. (2008) Date flesh: Chemical composition and characteristics of the dietary fibre. *Food Chemistry*, 111, 676–682.
- Gilani, S.J., Bin-Jumah, M.N., Al-Abbasi, F.A., Albohairy, F.M., Nadeem, M.S., Ahmed, M.M., Alzarea, S.I. & Kazmi, I. (2022) The ameliorative role of hibiscetin against high-fat diets and streptozotocin-induced diabetes in rodents via inhibiting tumor necrosis factor-α, interleukin-1β, and malondialdehyde level. *Processes*, 10, 1396.
- Hammouda, F.M., Rizk, A.M. & Abo-Zaid, M.A.M. (2013) Phytochemical study of *Balanites aegyptiaca* (Desert date) fruit pericarp. *Middle East. J. Appl. Sci.*, 3, 173–179.
- Harborne, J.B. (1998). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Edition*, Vol. 3. Springer.
- Al-Saaedi AM. Antipyretic Activity of the Aqueous Extract of Cumin (Cuminum cyminum L.) with Yeast Induced Pyrexia in Female Rats (2021). *Journal of Science*. University of Thi-Qar, 8, 33–35.
- Kazmi, I., Al-Abbasi, F.A., Afzal, M., Shahid Nadeem, M.S. & Altayb, H.N. (2023) Sterubin protects against chemically induced Alzheimer's disease by reducing biomarkers of inflammation-IL-6/IL-β/TNF-α and oxidative stress-SOD/MDA in rats. *Saudi Journal of Biological Sciences*, 30, 103560.
- Kokate, C.K., editor Practical Pharmacognosy (1994), 4th edn, *Vallabh Prakashan*:, 112, 120.
- Mukherjee, P.K. (2007). *Quality Control of Herbal Drugs*, 2nd edn, Business Horizons, pp. 2–14.
- Parkhe, G. & Bharti, D. (2019) Phytochemical investigation and determination of total phenols and flavonoid concentration in leaves extract of Vitex trifolia Linn. *Journal of Drug Delivery and Therapeutics*, 9, 705–707.