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Original Article

GC-MS Analysis and Assessment of Antioxidant and Anthelmintic Potential of the Ethanolic Root Extract of *Borassus flabellifer* Linn.

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ABSTRACT

Introduction: The present study was to investigate the phytocomponents by using GC-MS analysis, *in-vitro* antioxidant activity and the anthelmintic property of ethanolic extract of *Borassus flabellifer* Linn (EEBFL) root. **Methods:** EEBFL was prepared from roots of *B. flabellifer* by cold maceration. EEBFL was used to analyse phytocomponents by GC-MS and the *in-vitro* antioxidant activity was quantified using are low he ABTS method. Anthelmintic activity was studied on *Pheritima posthuma*. **Results:** The phytochemical investigation by GC-MS study identified 15 components in the extract of *B. flabellifer*. In antioxidant activity, the percentage scavenging range from 32.33% to 61.66% (IC_{50} 160±2.5) and by standard drug (ascorbic acid) 48.33% to 70.66% (IC_{50} 105±4.33) using the ABTS method. For anthelmintic activity, EEBFL showed paralysis time ranging from 107 min to 127 min and a death time ranging from 148 min to 177 min comparted to the standard drug Albendazole (paralysis time 94 min to 62 min and death time 124 min to 136 min on *Pheritima posthuma*). **Conclusion:** Ethanolic *B. flabellifer* Linn root extract is an effective agent in the management of antioxidant and anthelmintic activities due to presence of biologically active phytocomponents.

Key words: *B. flabellifer*, GC-MS study, ABTS, Ascorbic acid, *Pheritima posthuma*.

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INTRODUCTION

Medicinal plants are useful for the treatment of various diseases and have been used since ancestral times. Plants are an important resource of new biological compounds or natural molecules for treating illnesses. The natural products were well explored as a lead to discover new drug molecule with therapeutic potential and safety aspects.[1] Exploration of medicinal plants for curative purposes is mainly based on the available traditional information from the experts and local population.[2,3] The investigation of medicinal properties of various plants has attracted increasing interest recently due to their potent pharmacological activities, convenience to users, economic viability and low toxicity.[4,5]

In the last few years, gas chromatography mass spectrometry (GC-MS) has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species.[6,7] It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to elect crude plant extracts with potentially useful properties for further chemical and pharmacological investigations.[8] Antioxidant are compounds that trap free radicals, thus inhibiting the oxidative mechanisms that lead to degenerative disease.[9] Antioxidants may be either synthetic or natural and the latter is the focus for herbal remedies as they are obtained from medicinal plants.[10-12] Anti-helmintic are the agents that target the parasite and expel the worms (helminths) from the body either by stunning or killing them. In the recent years several studies has been conducted in synthetic chemistry for the anti-helminthic activity, but all of them shows toxic side effect. Therefore to achieve the therapeutic effect up to the level with non-toxic treatment for helminthiasis, the research on plant derivative is highly needed[13,14]

The plant *Borassus flabellifer* L, Kingdom: Plantae, Phylum: Spermatophyta, Subphylum: Angiospermae, Class: Monocotyledonae, Family: Arecaceae (Figure 1), mainly contains gums, albuminoids, fats, steroidal glycosides and carbohydrate including sucrose.[15] It also contains spirostane type steroids like borassosides and dioscin.[16] *B. flabellifer* has been reported for various pharmacological activities viz. methanolic root extract reported for antibacterial, antifungal and antioxidant activity.[17] Ethanolic fruit and seed extracts have been studied using phytochemical tests and the antioxidant and cytotoxic activities have been quantified.[18] Methanolic, ethanolic and aqueous seed extracts have antimicrobial activity.[19] Alcoholic root extract has hypoglycaemic activity[20] and methanolic leaves extract has anthelmintic activity.[21] Ethanolic flower extract has also been reported for anti-inflammatory activity,[22] ethanolic and aqueous seed extracts have diuretic activity,[23] whilst aqueous fruit extract has wound healing activity.[24]

A detailed literature review on *B. flabellifer* has shown that there are no published reports worldwide related to ethanolic root extract to date. Therefore, this study investigates the *in vitro* antioxidant, anthelminthic activity and the detection of phytocomponents using GC-MS (gas chromatography and mass spectrometry) analysis of the ethanolic extract of *B. flabellifer* Linn (EEBFL) root.

MATERIALS AND METHODS

Plant material

Based on the literature survey, one plant known as *Borassus flabellifer* was selected for the study. The roots of *Borassus flabellifer* Linn (family Arecaceae) was collected in the month of July, 2018 from various areas of Tallarevu mandal of Andhra Pradesh and authenticated by Dr. A. Satya Vani, Assistant Professor in Botany, S.T.P.P. Govt. Junior College, Yanam, with a authentication number STPP/SlNO/43/2018. A plant herbarium voucher specimen was prepared and preserved in the Department of Pharmacognosy, Koringa College of Pharmacy, Korangi, East Godavari Dist., Andhra Pradesh, India.

Preparation of ethanolic extract

The roots of *Borassus flabellifer* were extracted by cold maceration method. The roots were dried using shade drying method. Shade drying was carried out under natural air flow and surrounding's temperature (mean temperature 25°C) for 72 hr. The roots were cut into small pieces in a hand operated grinder and were grounded to coarse powder. 204 g of *Borassus* powder was mixed with 1200 ml of 100% ethanol (Analytical laboratory grade from B. S. Trading Co. Pvt. Ltd., Kolkata) in a conical flask and kept for 5 days. The mixture was filtered and filtrate was concentrated using rotary evaporator at 45-50°C and the resultant residue was kept in a refrigerator till further use.

Chemicals and Instruments

ABTS [2, 2' azino bis (3- ethylbenzthiazoline- 6- sulfonic acid) diammonium salt] was purchase from Sigma-Aldrich, Mumbai, India. Potassium persulphate, disodium hydrogen phosphate, sodium chloride and ethanol were purchase from Merck, Mumbai, India. The UV-Visible spectrophotometer of UV-1900, Shimadzu, Japan, with 1 cm match cells was used. The GC-MS, Thermo GC-TRACE ultra ver.: 5.0, Thermo MS DSQ II, from Thermo Fisher Scientific was used.

Phytochemical evaluation

A plant may be considered as a biosynthetic laboratory, not only for the chemical compounds such as carbohydrates, proteins and lipids that are utilized as food by man, but also for a multitude of a compounds like glycosides, alkaloids, volatile oils, saponins, etc., that exert a physiological effect. The compounds that are responsible for therapeutic effects are usually the secondary metabolites. A systematic study of a crude drug embraces a consideration of both primary and secondary metabolites derived as a result of plant metabolism. The ethanol extract of the roots of *B. flabellifer* were subjected to preliminary phytochemical screening[25] for the detection of various phytoconstituents.

GC-MS analysis of phytocomponents

The phytocomponent investigation of the ethanolic root extracts were performed on Thermo GC-TRACE ultra ver.: 5.0, Thermo MS DSQ II, (Thermo Fisher Scientific). Experimental conditions of the GC-MS analysis were as follows: TR 5-MS capillary standard non-polar column, dimension: 30Mts, ID: 0.25 mm, Film thickness: 0.25µm. Flow rate of the mobile phase carrier gas helium (He) was set at 1.0 mL/min. In the gas chromatography, the temperature programme (oven temperature) was set at 40°C raised and to 250°C at 5°C/min. The injection volume was 1 µl. Samples dissolved in chloroform were run at a range of 50–650 *m/z* and the results were compared by using Wiley Spectral library search programme.[26]

In-vitro antioxidant activity

The ABTS radical scavenging method involves the scavenging of ABTS [2, 2' azino bis (3- ethylbenzthiazoline- 6- sulfonic acid) diammonium salt] radical. ABTS and potassium per-sulphate react to produce the ABTS radical, a blue green chromogen.[27] In the presence of antioxidant reductant, the coloured radical is converted back to colourless ABTS and the absorbance was measured at 734 nm. The test sample was prepared by dissolving 100mg of dried extract in 100ml of ethanol/ distilled water (60 ml ethanol with 40 ml of distilled water) to make a stock solution of 1mg/ml, aliquots from this stock solution were further diluted with ethanol/distilled water to the concentration required. The phosphate buffer pH 7.4 was prepared using 0.238g of di-sodium hydrogen phosphate, 0.019g of potassium di-hydrogen phosphate and 0.8g of sodium chloride dissolved in 100ml of distilled water and the pH 7.4 was adjusted.

Then, 0.1ml of potassium per sulphate and 25ml of ABTS were mixed and used after 2 hr. To 1 ml of various concentrations (100 - 200 µg/ml) of the extracts, 0.6ml of ABTS radical cation and 3.4ml of phosphate buffer pH 7.4 were added. For the control purpose, ethanol was used instead of the test compound. The absorbance was measured at 734nm. The experiment was performed in triplicate.[27]

% Scavenging = (Control-Test/ Control) × 100

Evaluation of *in-vitro* anthelmintic activity

Indian adult earthworms (*Pheretima posthuma*) were used to carry out the evaluation of anthelmintic activity of ethanolic extract of the *B. flabellifer* root extracts. The earthworms were collected from the moist soil of Korangi, Andhra Pradesh. Worms were washed with saline water to remove the fecal matter and stored in Tyrode solution (sodium chloride 8g, potassium chloride 0.2g, calcium chloride 0.2g, sodium-di-hydrogen phosphate 0.1g, magnesium chloride 0.1g, glucose 1g and sodium bicarbonate 1g in 1000 ml distilled water). Worms about 9 cm length and 0.2-0.3 cm wide were selected for the experiment. The anthelmintic activity was evaluated according to the standard method[28] on the adult Indian earthworm *Pheritima posthuma*. The standard drug albendazole (99.60%, procured from Cadila pharmaceutical ltd, Ahmedabad, India) was diluted with normal saline solution and three concentrations of standard drug sample (25, 50 and 100 mg/mL) were poured into petri dishes. The ethanolic of *B. flabellifer* root extract was diluted with saline solution (0.90% w/v of NaCl) to achieve 25, 50 and 100 mg/mL concentrations. Saline solution (0.9% NaCl) alone used as the negative control. All dilutions were poured into the petri-dishes for testing. Seven sets of petri-dishes were taken and numbered. Six earthworms (n=6) of approximately equal size (8 cm) were placed in each petri-dish at room temperature. Then the paralysis and death (lethal) time was observed and noted for all petri- dishes and recorded in terms of minutes. The experiments were performed in triplicate. The anthelmintic property was evaluated by the observation of paralysis time (loss of movement) and death time (no movement) of the earthworm. The loss of movement of worm can be confirmed by using of normal saline solution. The death time of worm should be finalised by dipping in 50°C warm water. The colour was also evaluated and any fading of the colour of worm was noted.[28]

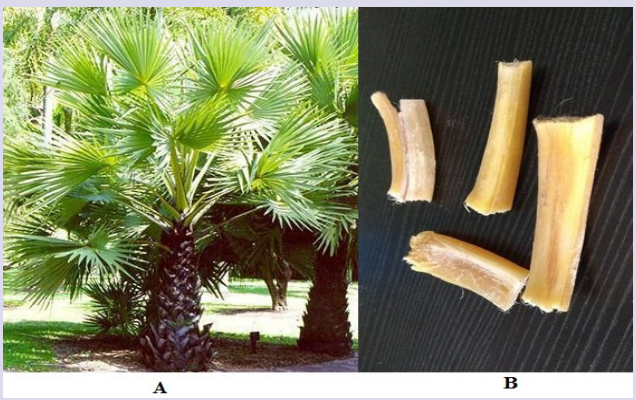

Figure 1: Image of the whole plant *Borassus flabellifer* Linn (A) and roots (B).

RESULTS

Preliminary phytochemical tests

The preliminary phytochemical tests of the ethanolic extract (roots) of *B. flabellifer* indicates the presence of steroids and terpenoids, saponins, phenols, tannins and flavonoids and absence of alkaloids, carbohydrates, glycosides, proteins and amino acids.

Determination of molecular weight and molecular formula of the phytocomponents by GC-MS

The various compounds present in the ethanolic extract of *B. flabellifer* roots were identified using mass spectrometry in conjunction with GC (GC-MS). The GC-MS chromatogram (Figure 2) indicates the presence of a variety of components with various retention times. The components were eluted at different times (analysed by the mass spectrometer), indicating difference in their structure and physiochemical properties. A large compound can split into smaller components resulting in peaks appearing at different *m/z* ratios (as shown in Figure 2). The compounds corresponding to the peaks obtained from components were established from the data library. In the extracts of *B. flabellifer,* 15 biomolecules were identified and their molecular weight and formula were determined. The list of phytoconstituents is tabulated in the Table 1 and chemical structure are represented in Figure 3.

In-vitro antioxidant activity

In-vitro antioxidant activity of ethanolic extract (roots) of *B. flabellifer* was measured at two different concentration levels. The antioxidant activity was measured by percentage scavenging and the concentration range was tested at 100µg/ml and 200µg/ml. For the ethanolic extract (roots) of *B. flabellifer,* the percentage of scavenging were as follows: For the range 100µg/ml to 200µg/ml, the percentage of scavenging was between 32.33 to 61.66 % (IC_{50} value 160±2.5) and the percentage of scavenging for standard was between 48.33 to 70.66% (IC_{50} Value 105±4.33). The reaction of $ABTS^{+}$ with free radical scavengers present in the test sample occurs rapidly and is assessed by following the decrease in sample absorbance in 734nm. The results are tabulated in the Table 2

Table 1: The identified compounds in the ethanolic extracts of *Borassus flabellifer* roots.

Compounds	Retention Time (Minute)	Compound name	Molecular formula	Molecular weight	Peak Area%	Compound Nature
A	8.07	Dimethyl derivative of vitamin D3-triol	$C_{28}H_{48}O_3$	432	0.92	Steroid
B	9.33	7,8-Bis (trimethylsilyl) benzo (5,6-g)-1H,3H-quinazoline-2,4-dione	$C_{18}H_{24}N_2O_2Si_6$	356	1.89	Heterocyclic
C	12.61	1-Propanone, 2-bromo-1-phenyl-(CAS)	C_9H_9BrO	212	1.44	Ketone
D	14.34	Acrylic acid, 2-Bornyl ester	$C_{13}H_{20}O_2$	208	3.78	Ester
E	17.12	2-tert-Butyl-4-trifluro methyl-1-methyl imidazole	$C_9H_{13}F_3N_2$	206	0.49	Heterocyclic
F	17.71	Tricyclo[4.2.1.1(2,5)] dec 7-en-9-ol	$C_{10}H_{14}O$	150	6.27	Alcohol
G	20.57	Irgacure 184	$C_{13}H_{16}O_2$	204	13.71	Ketone
H	22.99	1-Bromo-1,4,4a,5,8,8a-hexahydronaphthalene	$C_{10}H_{13}Br$	212	32.73	Aromatic
I	25.71	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.75	Ester
J	37.14	2,9-bis(2',6'-dimethoxy phenyl)-1,10-phenanthroline	$C_{28}H_{24}N_2O_4$	452	1.36	Aromatic
K	38.58	Morphinan,7,8-didehydro-4,5-epoxy-3,6-bis[(trimethylsilyl) oxy]-(5a,6a)	$C_{22}H_{33}NO_3Si_2$	415	3.65	Heterocyclic
L	44.43	Propanoic acid	$C_{27}H_{42}O_4$	430	2.15	Carboxylic acid
M	45.09	Methyl 2-bromomethyl-10-tetrahydropyranyloxy-2-decenoate	$C_{17}H_{29}BrO_4$	376	7.84	Carboxylic acid
N	46.50	Tetrasiloxane,decamethyl	$C_{10}H_{30}O_3Si_4$	310	1.68	Alkane
O	48.53	4-8-Bis(2-propyl-amino)-2,6-dichloro-1,5-naphoquionone	$C_{16}H_{18}C_{12}N_2O_2$	460	1.87	Ketone

Table 2: Results for *in-vitro* antioxidant activity of ethanolic extract of *Borassus flabellifer*.

Concentration (µg/ml)	% Scavenging of ethanolic extract	% Scavenging of Standard
100	32.33±0.57	48.33±1.15
200	61.66±1.52	70.66±2.08
IC_{50}	160±2.5	105±4.33

Std- Ascorbic acid. Readings are calculated ± S.E.M, n = 3.

and Figure 4.

Evaluation of *in-vitro* anthelmintic activity

The ethanolic extract of *B. flabellifer* roots were tested at three different concentrations against on *Pheritima posthuma* (Indian adult earthworms) for *in-vitro* anthelmintic activity. The paralysis time and death time were compared with reference to standard drug albendazole. The paralysis time of earth worms ranged from 107 min to 127 min for the *B. flabellifer* extracts and 62 min to 94 min for the standard drug

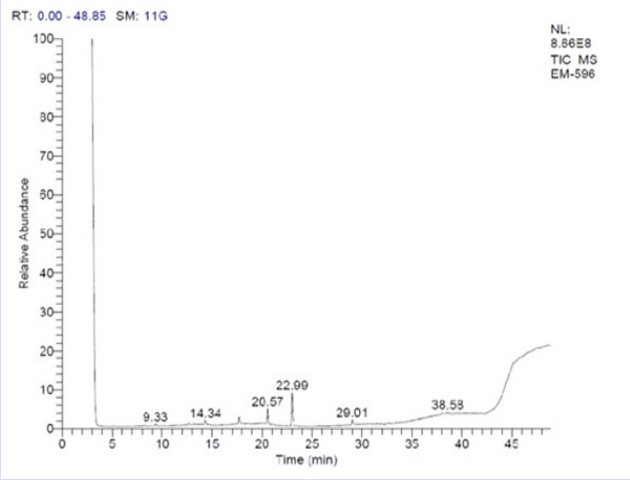

Figure 2: GC-MS chromatogram of ethanolic extracts of *Borassus flabellifer* roots.

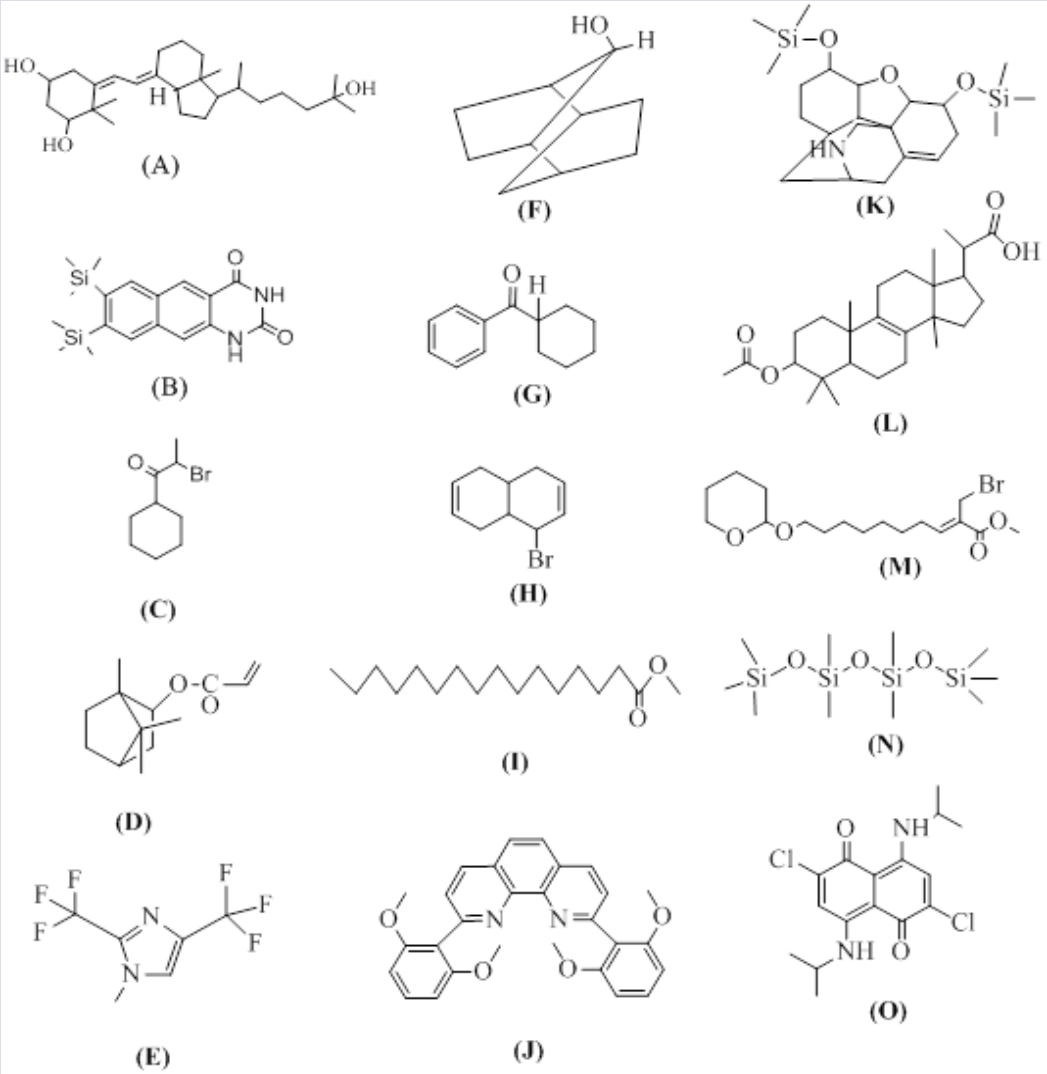

Figure 3: Structural representation of compounds (A-O) identified in the EEBFL roots by GC-MS.

Table 3: Results of *in-vitro* anthelmintic effect of EEBFL roots against *Pheritima posthuma*.

Treatment	Concentration (mg/mL)	Paralysis Time(min)	Death Time (min)
Albendazole (Standard)	25	94±5.29	136±3.78
	50	79±5.56	129±4.00
	100	62±4.00	124±3.46
Borassus flabellifer extract	25	127±5.56	177±3.60
	50	119±1.73	155±4.35
	100	107±2.64	148±2.00
Control (Saline solution)	0.9 % Nacl	No paralysis	No death

±SD value, *n*=6.

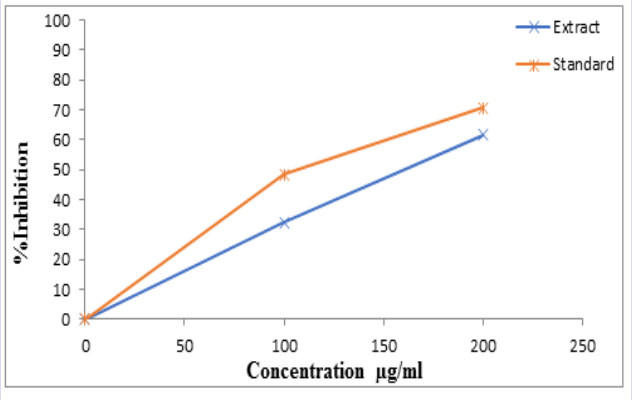

Figure 4: Graphical representation of *in-vitro* antioxidant activity of EEBFL.

albendazole, whereas the death time of earth worms ranged from 148 min to 177 min for the *B. flabellifer* extracts and 124 min to 136 min for albendazole when tested at different concentration of these extracts and standard drug ranging from 25 to 100 mg/mL. The results are presented in Table 3.

DISCUSSION

The preliminary phytochemical screening tests of *B. flabellifer* revealed the presence of saponins, tannins, flavonoids, glycosides and terpenoid. The preliminary screening tests may be useful in the detection of the bioactive compounds and subsequently may lead to drug discovery and development.[29] The phytocomponent evaluation was performed by GC-MS and the biological evaluation includes antioxidant activity test by ABTS method and anthelmintic activity test against *Pheretima posthuma*. The different pharmacological activities of *B. flabellifer* root extract can be described by investigating the chemical composition of the extract using GC-MS analysis. The 15 phytocomponents investigation by GC-MS study were identified in the extract of *B. flabellifer* as Dimethyl derivative of vitamin D3-triol, 7,8-Bis (trimethylsilyl) benzo (5,6-g)-1H,3H-quinazoline-2, 4-dione, 1-propanone, 2-bromo- 1-phenyl-(CAS), acrylic acid, 2-bornyl ester, 2-tert-butyl-4-trifluromethyl-1-methyl imidazole, tricyclo[4.2.1.1(2,5)] dec 7-en-9-ol, irgacure 184, 1-bromo-1,4,4a,5,8, 8a-hexahydronaphthalene, hexadecanoic acid, methyl ester, 2,9-bis(2',6'-dimethoxyphenyl)-1, 10-phenanthroline, morphinan,7, 8-didehydro-4, 5-epoxy 3, 6 bis [(trimethylsilyl)oxy] (5a,6a), propanoic acid, haloxazolam, 4-8-bis(2-propyl-amino)-2,6-dichloro-1,5-naphoquionone. Further research study may lead to the isolation of further phytocomponents and their structure elucidation will be helpful for further drug development.

The result of *in-vitro* antioxidant activity test of the extract was measured by ABTS method at two different concentrations (100µg/ml and 200µg/ml). A concentration dependant effect was observed in this antioxidant experiment. Higher concentrations of the extract were more effective in quenching the free radical system. The results revealed that ethanolic extract has a potent antioxidant activity which may attributed to the numerous phenolics. Flavonoids, tannins, catechins and other phenolics are the examples of common plant metabolites having prominent antioxidant activity.[30]

The investigation of anthelminthic activity of the ethanolic extract of *B. flabellifer* showed the paralysis time and death time inversely proportional to the concentration of extract. The paralysis and death time of earth worms were very significant when compared with the standard drug albendazole. There was a significant decrease in the value of paralysis time and death times as the concentration of the extracts and standard drug albendazole were increased against *P. Posthuma*. The various phytochemical studies suggest that the presence of tannins and saponins which are may be responsible for anthelmintic property.

CONCLUSION

Due to the undesirable side effects of synthetic drugs, phytocomponents of medicinal plant obtained in the form of extract may be useful for drug discovery and development against various diseases. The presences of phytoconstituents in the plant extract were confirmed through GC-MS analysis. The ethanolic extract of the plant showed significant antioxidant and anthelmintic activity due to presence of more amounts of antioxidant and anthelmintic compounds (phytochemicals or phytocomponents). Thus, the *B. flabellifer* Linn. roots could be considered as phytopharmaceutical importance of natural antioxidant and anthelmintic agent.

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CONFLICT OF INTEREST

The authors does not have any conflict of interest.

ABBREVIATIONS

GCMS: Gas chromatography-mass spectrophotometry; **ABTS:** 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); **UV:** Ultra Violet; **SEM:** Standard error mean; **NaCl:** Sodium chloride; **EEBFL:** Ethanolic extract of *B. flabellifer* Linn.

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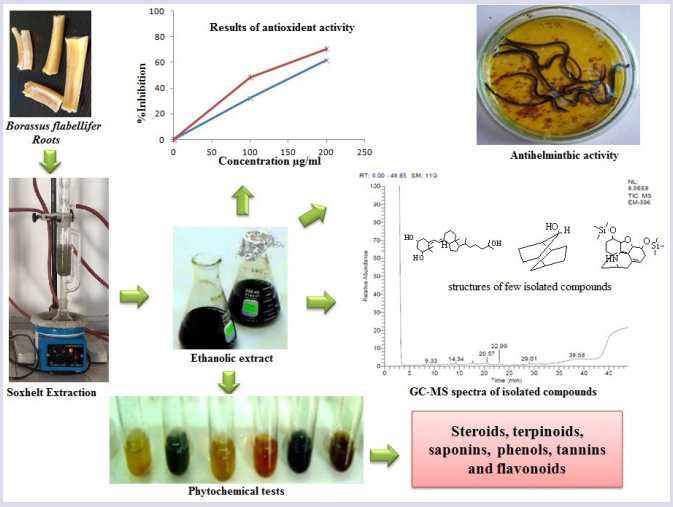
PICTORIAL ABSTRACT



SUMMARY

The present study was to investigate the phytocomponents by using GC-MS analysis, *in-vitro* antioxidant activity and the anthelmintic property of ethanolic extract of *Borassus flabellifer* Linn. (EEBFL) root. The extract was prepared from roots of *B. flabellifer* by cold maceration and was used to analyse phytocomponents by GC-MS and the *in-vitro* antioxidant activity was quantified. The phytochemical investigation by GC-MS study identified 15 components in the extract of *B. flabellifer*. In antioxidant activity, the percentage scavenging range from 32.33% to 61.66% (IC_{50} 160±2.5) and by standard drug (ascorbic acid) 48.33% to 70.66% (IC_{50} 105±4.33). Ethanolic *B. flabellifer* Linn. root extract is an effective agent in the management of antioxidant and anthelmintic activities due to presence of biologically active phytocomponents.

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