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Research Article

GAS CHROMATOGRAPHY - MASS SPECTROMETRY ANALYSIS, INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY INVESTIGATION, AND ANTIMICROBIAL SCREENING OF *CAESALPINIA BONDUCELLA* (L.) ROXB SEED KERNEL

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ABSTRACT

Objective: The objective of this study was to determine heavy metals using inductively coupled plasma mass spectrometry (ICP-MS) and phytochemical constituents of methanol extract of *Caesalpinia bonducella* seed kernel using gas chromatography - mass spectrometry (GC-MS).

Methods: In GC-MS investigation, 95% methanol extract of *C. bonducella* seed kernel was performed on JEOL GC MATE II, column HP 5 MS, and Quadruple, double-focusing mass analyzer. Determination of heavy metals from *C. bonducella* powder was performed using Thermo scientific I CAP Q instrument, for plant powder digestion Anton Paar Microwave model: Multiwave 300 was used. Antimicrobial activity was evaluated using microdilutions broth method.

Results: GC-MS analysis of methanol extract of *C. bonducella* seed kernel has shown 11 different phytoconstituents. ICP-MS analysis has shown the concentration of ten heavy metals in *C. bonducella* seed kernel as follows: Cr 1.5 ppm, Fe 72.72 ppm, Co 0.33 ppm, Ni 3.03 ppm, Cu 10.73 ppm, Zn 18.44 ppm, As 0.03 ppm, Cd 0.02 ppm, and Pb 0.58 ppm.

Conclusion: Result showed that the plant is safe to use from toxic heavy metals such as As, Cd, and Pb. It is suitable to check frequently the heavy metal content in the plant used in traditional medicines before given to patients.

Keywords: Heavy metals, *Caesalpinia bonducella*, Inductively coupled plasma mass spectrometry, Gas chromatography - mass spectrometry.

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INTRODUCTION

Medicinal plants have been used for the treatment of various diseases from ancient time and have been playing an important role in the discovery of the modern day medicines with novel chemical constituents [1]. In the U.S.A., about 8% of hospital admissions are due to side effects of synthetic drugs [2]. Nowadays, the use of herbal medicines for the treatment of various diseases expands continuously because people believe that herbal medicines have no toxic effects and they are safe to use [3]. Medicinal plants contain both organic and inorganic constituents [4]. Heavy metals are those metals which have relative atomic number > Na and density > 5 g/cm^3 [5]. Plants need some of the heavy metals for their structural and biological functions such as Zn, Cu, Ni, Mn, Cr, and Fe; these elements are considered as essential trace elements [6] whereas toxic elements which have unknown biological role in the plant such as Cd, As, Hg, and Pb are toxic even at low concentration [7]. Heavy metals are present in medicinal plants in different concentrations, and these levels are influenced by several factors such as type of plant, the type of soil, the climate, and the type of agriculture [8]. The human body requires trace elements in very minute quantities to maintain good health, proper body functions, and development [9,10]. It gets translocated to human through the use of medicinal plants grown in contaminated environments, in which, soils, water, and air contain high concentration of heavy metals [11-14].

C. bonducella (L.) Roxb is belonging to the caesalpiniaceae [15], commonly known as Nata Karanja [16]. It is found throughout India and tropical countries in the world [17]. "Bonducella" is derived from the Arabic word "Bonduce" the meaning is "Little ball" [18]. There are several reports on phytochemical constituents of *C. bonducella* (L.) [17,19-21], but no work on metal constituents is reported earlier. The aim of this study is to determine heavy metals of *C. bonducella* (L.) Roxb seed kernel and to ensure its safe usage in herbal medicine.

METHODS

Collection of plant material: The *C. bonducella* seeds were purchased from local vendor at Aurangabad, India. It was authenticated by Dr. Narayan Panddhura, Department of Botany and Dr. B.A.M University – Aurangabad. Seeds were broken, and kernel from outer seed shell was separated.

Preparation of extract: Seed kernel of *C. bonducella* was ground to powder form and extracted with 95% methanol in a Soxhlet extractor for 6 h; further, the extract was filtered and concentrated on rotary vacuum evaporator. Sticky, white extract was kept in an amber bottle in refrigerator for further use.

GAS CHROMATOGRAPHY - MASS SPECTROMETRY (GC-MS)

GC-MS investigation of methanol extract of seed kernel of *C. bonducella* was carried out under the following conditions: Instrument name: JEOL GC MATE II, column HP 5 MS, and Injector T 220°C; carrier gas: High pure helium; flow rate: 1 ml/min, oven temperature 50–250 at 10°/min, and Ion chamber at 250°C.

ICP-MS

Microwave digestion

Plant powder digestion was carried out using Anton Paar Microwave model: Multiwave 300. Plant powder (0.1 g) was dissolved in 7 ml HNO_3 (37%, Merck) and 1 ml H_2O_2 (30%, Merck), and the vessel was immediately closed to avoid contamination. The sample was hold for 25 min., zero ramp time at 400 watts and then retained for 30 min., 5 ramp at 500 watts. After cooling, the vessel was opened, and the solutions were diluted to 100 ml with Milli Q water (Thermo scientific, Barnstead, smart 2 pure). The vessel was closed and shaken thoroughly to complete the dissolution.

Elemental measurement using ICP-MS

ICP-MS instrument (iCAP Q, Thermo fisher scientific, USA) was used for measuring the concentration of elements in the digested sample solution under the following conditions: Plasma RF forward power 1548.6 W, sample uptake: 30 s, cool flow read back: 13.67 L/min, nebulizer flow: 1.0180 L/min, auxiliary flow read back: 0.796 L/min, integration time: 10 s, peristaltic pump speed: 40 rpm, sampler and skimmer cones: Ni, and analysis mode: eQuant.

Chemical reagents and analytical method

Deionized water of 18.2 megaohm was used for dilution and sample preparation. The external calibration solutions were prepared from standard certified multielements solutions (Merck, USA). Stock solution was prepared from a mixture of 30 elements at concentration of 10 ppm from Merck. A concentration of 25 µg/l of yttrium (Y) or iridium (Rh) was used as internal standard for the analysis. Y was used for low mass elements such as Cr, Co, Cu, and Zn, whereas Rh was used for high mass elements such as Cd and Pb. A tune solution (BICAP) containing Li, Co, In, and U was used for performance validation and instrument tuning, which contains 1 µg/l of each element in 2% HNO_3 and 0.5% HCl.

Evaluation antibacterial and antifungal activity

Microbroth dilution method was used to determine the minimum inhibitory concentration (MIC) of methanol extract of *C. bonducella* seed kernel. Four strains of gram bacteria were used; two Gram-positive bacteria (*Staphylococcus aureus* MTCC 96 and *Streptococcus pyogenes* MTCC 442) and two Gram-negative bacteria (*Escherichia coli* MTCC 443 and *Pseudomonas aeruginosa* MTCC 1688), for fungi: *Candida albicant* (MTCC 227), *Aspergillus niger* (MTCC 282), and *Aspergillus clavatus* (MTCC 1323) were used. All MTCC cultures were collected from Microcare Laboratory, Surat. Ampicillin, chloramphenicol, ciprofloxacin, and norfloxacin were used as standard antibacterial drugs, whereas nystatin and griseofulvin were used as standard antifungal drugs. Test microorganisms and growth media, sample preparation, and MIC were determined as standard method [22].

RESULT AND DISCUSSION

GC-MS investigation

GC-MS analysis result Figure 1 of methanol extract of *C. bonducella* seed kernel (MCBSK) was showed 11 peaks indicating the present of 11 different phytoconstituents. The library search was indicated the presence of following compounds in this extract: 2,4{1H,3H}-pyrimidinedione, dihydro-3-methyl, benzene,{1-methylenebutyl}-, benzidine, á-neoclovene, flavone, 3,6-non-adienedioic acid, 5,5-dimethyl dimethyl ester, 9,12-octadecadienoic acid{Z,Z}, octadecanoic acid, 3-oxo, methyl ester, isopropyl stearate, 9,12 octadecadienoic acid {Z,Z},2,3-dihydroxypropyl ester, 9,12,15-octadecatrienoic acid, and 2,3-dihydroxypropyl ester {Z,Z,Z}.

Photochemical constituents' retention time value, area, area %, peak height, molecular formula, molecular weight, and identified compound name are shown in Table 1. The results were revealed that 9,12-octadecanoic acid (Z, Z) (23.953%) at RT (19.67); octadecanoic acid, 3-oxo, and methyl ester (11.542%) at RT (21.28%); flavone (11.09%) at RT (17.75); and 3,6-non-adienedioic acid and 5,5-dimethyl dimethyl ester (9.99%) at RT (18.7) were found as a major components of MCBSK. We found that the majority of components were fatty acids and their derivatives. Total area percentage of six fatty acids or their derivatives (RT 18.7, 19.67, 21.28, 22.92, 24.35, and 25.3) is 71.36%. 9,12-octadecanoic acid (Z,Z) has been reported to have many biological activities such as anti-inflammatory, hypercholesterolemic activity,

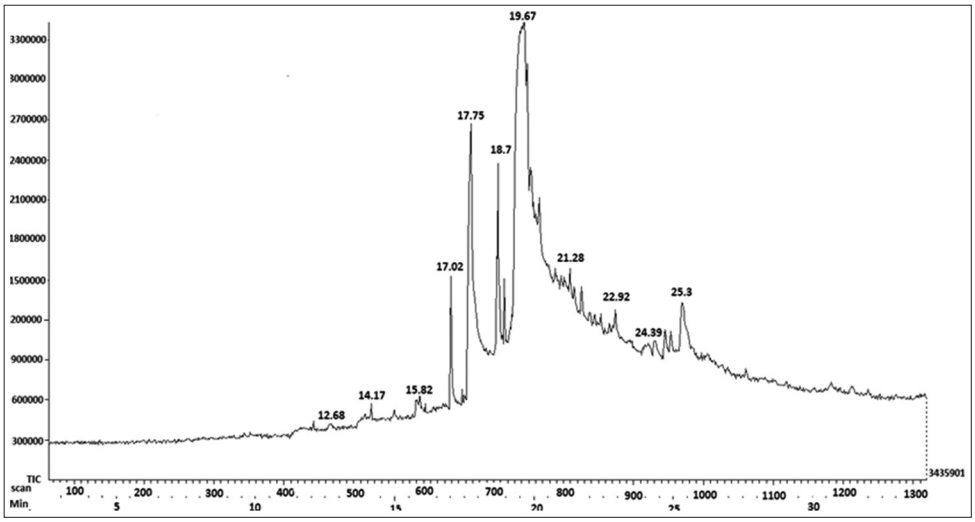

Fig. 1: Gas chromatography - mass spectrometry chromatogram of methanol extract of *Caesalpinia bonducella* seed kernel

Table 1: Phytochemicals compounds identified in methanol extract of *Caesalpinia bonducella* seed kernel

S. No.	R.T (Min)	Peak area	Area %	Height	Molecular weight (g/mol)	Chemical formula	Probable compound
1	12.68	8608645	3.149	420082	128	$C_5H_8N_2O_2$	2,4{1H,3H}-pyrimidinedione, dihydro-3-methyl
2	14.17	10298439	3.77	570956	146	$C_{11}H_{14}$	Benzene, {1-methylenebutyl}-
3	15.82	11758505	4.302	604553	184	$C_{12}H_{12}N_2$	Benzidine
4	17.02	14631809	5.353	1524914	204	$C_{15}H_{24}$	á-neoclovene
5	17.75	30300375	11.09	2692186	222	$C_{15}H_{10}O_2$	Flavone
6	18.7	27304042	9.99	2371347	238	$C_{14}H_{22}O_3$	3,6-Non-adienedioic acid, 5,5-dimethyl dimethyl ester
7	19.67	65474434	23.953	3435901	280	$C_{18}H_{32}O_2$	9,12-octadecadienoic acid {Z, Z}
8	21.28	31550935	11.542	1565542	312	$C_{19}H_{36}O_3$	Octadecanoic acid, 3-oxo, methyl ester
9	22.92	24741051	9.051	1276576	324	$C_{21}H_{42}O_2$	Isopropyl stearate
10	24.35	21560304	7.89	1060147	354	$C_{21}H_{38}O_2$	9,12-octadecadienoic acid {Z, Z}-, 2,3-dihydroxypropyl ester
11	25.3	24422286	8.934	1329418	352	$C_{21}H_{36}O_4$	9,12,15-octadecatrienoic acid, 2,3-dihydroxypropyl ester {Z, Z, Z}

cancerpreventive,hepatoprotective,anti-acne,antiarthritic,anticoronary, antiandrogenic, and antihistamine [23]. 9,12,15-octadecatrienoic acid and 2,3-dihydroxypropyl ester {Z,Z,Z] have been reported to have anti-inflammatory and central nervous system depressant activity [24].

ICP-MS

The result of ICP-MS study of CBSK is summarized in Table 2. This study was designed to investigate the concentration of ten heavy metals (Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Pb, and Cd). The result shows that the concentrations of heavy metals of CBSK range between 0.02 ppm for Cd and 72.72 ppm for Fe, and the order of concentration of these ten heavy metals is Fe > Mn > Zn > Cu > Ni > Cr > Pb > Co > As > Cd.

Increase in use of fertilizers and other chemicals to meet the higher demands of food production or human consumption [25]; industrial activities and transportation activities lead to increase of heavy metals pollution. Numerous studies have investigated the presence of toxic contaminants in medicinal plants. The World Health Organization (WHO) recommends that medicinal plants should be tested for heavy metals content before exporting, and heavy metals should remain within permissible limits [26]. Fe is an essential element for the human body and plays essential function in the production of hemoglobin and in the oxygenation of red blood cell [27]. Fe concentration was observed in this study at 72.72 ppm with the highest concentration compared to other heavy metals concentrations. The WHO has not established the limits of Fe in medicinal plants and Fe concentration in CBSK higher than the permissible limit set by (Food and Agriculture Organization) FAO/WHO in edible plants (20 ppm) [28]. Fe deficiency causes anemia [29]. Cu is very important with Fe for the synthesis of hemoglobin and in regulation of immune function [30], is a component of several enzymes [31], is required for maintaining a health of heart and blood vessels [32], and is also required for antioxidant protection [33]. Cu was found in this plant 10.73 ppm, the WHO permissible limits of Cu in medicinal plants have not established, but national limits of Cu in Singapore for herbal medicines and products are 150 ppm [34]. Zn plays an important role in various cell processes [35] and has following three critical functions: Catalytic (Zn can activate 300 enzymes), structural (Zn plays a role in maintaining protein structure and DNA), and regulatory [36]. Zn concentration in this present study was observed 18.44 ppm, which is below the permissible limit (27.4 ppm) reported by FAO/WHO in edible plants. Mn is very important for several enzymatic processes and in regulation of immune responses of the body by breakdown of amino acids [37]. Mn concentration in this study was observed (19.77 ppm), which is higher than the limit of Mn in edible plants (2 ppm) set by FAO/WHO (1984). The WHO has not established any limits for Mn in medicinal plants. Ni is required by the human body in trace quantity and plays an important role in the production of insulin in the pancreas. Nickel has been reported to cause many diseases in high concentration such as lung cancer, nasal, renal disorder, dermatitis, sinus, chronic bronchitis, acute respiratory, and pulmonary fibrosis [38]. The WHO has not established limit for Ni in medicinal plants; however, the limit for Ni in edible plants has been set by FAO/WHO 1.63 ppm. Concentration of Ni in this study was observed at 3.03 ppm. Co is required in trace quantity for human body and is a key constituent of cobalamin known as Vitamin B_{12} [39]. The concentration of Co in the present study was observed at 0.33 ppm, and WHO has not established yet permissible limits for Co in medicinal plants. Cr plays an important role in glucose metabolism, maintaining the configuration of RNA and several enzymes can be activated by Cr [40], and Cr also is a vital component for insulin to stabilize blood sugar energy [41]. Cr concentration was observed 1.5 ppm in this plant under study, which is below WHO limits in national Canada for herbal material 2 ppm. As, Cd, and Pb are toxic metals and have no functions in the human body. Several countries including India, China, Canada, Thailand, Malaysia, and Singapore have established national permissible limits for Cd, As, and Pb in raw herbal material. In India, the permissible limits for Cd, As, and Pb in herbal material are 0.3 ppm, 3 ppm, and 10 ppm, respectively, according to AYUSH [42]. In the present study, the concentration of

Table 2: Concentration of heavy metals in seed kernel of *Caesalpinia bonducella*

S. No.	Element symbol	Atomic weight (g/mol)	Concentration (ppm)
1	Cr	51.996	1.50
2	Mn	54.938	19.77
3	Fe	55.845	72.72
4	Co	58.933	0.33
5	Ni	58.693	3.03
6	Cu	63.546	10.73
7	Zn	65.390	18.44
8	As	74.922	0.03
9	Cd	112.411	0.02
10	Pb	207.200	0.58

Table 3: Minimal inhibition concentration of methanol extract of *Caesalpinia bonducella* seed kernel

MIC (µg/ml)					
S. No.	**Extract code**	***Escherichia coli* MTCC443**	***Pseudomonas aeruginosa* MTCC1688**	***Staphylococcus aureus* MTCC96**	***Streptococcus pyogenes* MTCC442**
1	MCBSK*	250	500	125	100
Standard Antibiotic					
2	GEN	0.05	1	0.25	0.5
3	AMP	100	-	250	100
4	CMP	50	50	50	50
5	CIP	25	25	50	50
6	NOR	10	10	10	10

*MCBSK: Methanol extract of *Caesalpinia bonducella* seed kernel, GEN: Gentamicin, AMP: Ampicillin, CMP: Chloramphenicol, CIP: Ciprofloxacin, NOR: Norfloxacin, MIC: Minimum inhibitory concentration

Table 4: Antifungal activity of methanol extract of *Caesalpinia bonducella* seed kernel

Minimal fungicidal concentration (µg/ml)				
S. No.	**Extract code**	***Candida albicants* (MMTCC227)**	***Aspergillus niger* (MTCC282)**	***Aspergillus clavatus* (MTCC1323)**
1	MCBSK	250	>1000	>1000
Antifungal standard drugs				
2	NYSTATIN	100	100	100
3	Griseofulvin	500	100	100

MCBSK: Methanol extract of *C. bonducella* seed kernel

Cd, As, and Pb was found to be within permissible limits of AYUSH 0.02 ppm, 0.03 ppm, and 0.58 ppm, respectively.

Antibacterial and antifungal

The methanol extract of *C. bonducella* seed kernel is screened for antibacterial and antifungal activities. The results are shown in Tables 3 and 4, respectively. It is observed that in the case of positive bacteria *S. aureus*, the extract shows high inhibition compared to standard drug (ampicillin) and less inhibition compared to other standard drugs. In the case of positive bacteria *S. pyogenes*, the extract shows moderate inhibition similar to the inhibition of standard drug (ampicillin). For antifungal activity, the extract shows moderate inhibition compared to inhibition of standard drug nystatin and high inhibition compared to inhibition of standard drug (griseofulvin).

CONCLUSION

Toxic heavy metals of As, Cd, and Pb concentrations were found within Indian national permissible limits (AYUSH), whereas Ni, Cr, Cu, and Fe concentrations are exceeded permissible limits in edible plants. GC-MS analysis of 95% methanol extract of *C. bonducella* seed kernel revealed that 11 different phytoconstituents are present, and the majority of these phytoconstituents are fatty acids or their associated derivatives. The methanol extract possesses good to moderate antibacterial and antifungal activities.

AUTHORS' CONTRIBUTION

Ali Alrabie and Ola Basa'ar carried out the experiment and wrote the manuscript. Dr. Mazahar Farooqui, the research supervisor conceived the original idea, supervised the project, and corrected the scientific content of the manuscript.

CONFLICT OF INTERESTS

The authors have no conflicts of interest.

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