TITLE: CHARACTERIZATION OF FATTY ACIDS AND ANTIOXIDANT POTENTIAL ANALYSIS IN BAHUNIA RACIMOSA AND CASSIA FISTULA SEEDS OIL

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ABSTRACT

Physical and chemical characteristics, fatty acid composition, antioxidant potential and total phenolic content of oil extracted from Bahunia racimosa and Csasia fistula seeds are presented in this work. The results indicate that B. racimosa and C. fistula seeds constituted 27% and 44% oil respectively. The refractive index, saponification value and iodine value of B. racimosa and C. fistula seed oils were found to be 1.425 and 1.413, 125.6 and 105, 73 and 69 respectively. Prior to GC and GC-MS analysis, the extracted oil underwent methyl esterification. The major fatty acids reported here, in B. racimosa and C. fistula seeds oil, are Myristic acid (1.3% and 0.8%), Palmitic acid (17.5% and 19.3%), Stearic acid (11.2% and 7.3%), Oleic acid (11.8% and 18.1%), Linoleic acid (55.3% and 52.2%) and Linolenic acid (1.4% and 1.1%). The antioxidant potential were analyzed by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and Ferric reducing ability of plasma (FRAP) methods. The results confirmed that both the species have significant antioxidant properties. The total phenolic content in B. racimosa and C. fistula were 22.46±1.8 mg GAE/g oil and 19.82±1.2 mg GAE/g respectively. The oil from B. racimosa and C. fistula seeds could find new uses as a result of this study.

Keywords: *B. racimosa, C. fistula, fatty acids composition, antioxidant activity and total phenolic content.*

INTRODUCTION:

Degenerative diseases include mutagenesis, cancer, cardiovascular disease, and ageing are all brought about by the discharge of free radicals in biological systems. As a class of chemicals, antioxidants scavenge free radicals by blocking their formation, movement, and elimination throughout various stages of the oxidative process [1]. Traditional remedies derived from plants are now being used globally to treat a range of serious illnesses. Less harmful and economically viable, around 25% of medications are now derived from plant active components [2].

It has been claimed that several plants possess antioxidant properties. The root reason of investigating powerful antioxidant compounds derived from plants is the development of drug-resistant bacteria and oxidative stress-induced disorders such as cancer, cardiovascular disease, diabetes, atherogenesis, alzheimer's disease, and Parkinson's disease [3]. The therapy of some disorders has made extensive use of synthetic antioxidant compounds in recent decades, many of which have been shown to have a variety of harmful side effects. To avoid these harmful side effects, research into antioxidant compounds derived from plants is urgently needed. The Bauhinia racemosa Lam. (B. racemosa), is characterized by drooping branches. This plant may be found all over the world, including in China, the US, Ceylon, and India. Headaches, fevers, skin conditions, blood disorders, dysentery, and diarrhoea may all be alleviated with the use of B. racemosa's sweetish and astringent barks and leaves. A decoction of the bark of B. racemosa is suggested as an effective wash for ulcers; it is one of the most significant herbal medicines. Rumor has it that the bark may protect the liver and fight free radicals. The analgesic, antipyretic, anti-inflammatory, antispasmodic, and anthelmintic effects were shown by a leaf extract. The Ayurvedicpractise of using the tree to treat early-stage cancer is based on its anti-cancer properties [4]. Lupeol, betulin, β-sitosterol, and tetracyclic 2, 2-dimethylchroman are unique compounds found in the root of B. racemosa [5]. Flavonoids, crude protein, and fat are all present in the seed [6]. Plant components include ß-sitosterol and ß-amyrin in the bark, flavonols (kaempferol, quercetin) and coumarins in the leaves [7]. Cassia fistula Linn, a member of the Caesalpiniaceae family and often known as Amulthus or Indian laburnum, has a long history of medicinal use in many cultures' traditional medicine systems, particularly in Ayurveda, Unani, and Chinese medicine. Rhein, triterpenes, sugar, and potassium are among the many components found in C. fistula, which is both native to and grown in different parts of the globe. Animal studies have shown that C. fistula and its components may modulate biological activity, which can lead to disease management. C. fistula has been shown to have antimicrobial and antifertility [8] properties in many investigations. [9,10] According to research, there is substantial hepatoprotective action in the water-based extract of C. fistula fruit pulp. [11] Past research has shown that extracts from the bark of C. fistula may significantly reduce free radicals by blocking the lipid peroxidation that CCl_4 and $FeSO_4$ can induce in rat liver and kidney homogenates. [12] Experimental evidence suggests that C. fistula may be useful in treating infected wounds; rats given the treatment demonstrated enhanced wound closure and tissue regeneration. [13] The extensive history of C. fistula's significance in disease management may be traced back to its strong antioxidant content. A recent study was conducted to assess the antioxidant potential and protective effect of C. fistula Linn. on erythrocytes damaged by hydrogen peroxide. The results showed that the ethanolic extract of C. fistula had a high level of antioxidant activity and protected the cells from oxidative damage by more than 90%, while the aqueous extract of C. fistula only showed antioxidant and protective activity of 75%. [14] Due to the lack of prior research on the antioxidant properties of oil extracts from B. racemosa and C. fistula seeds, the purpose of the present study was to evaluate the antioxidant activities and also total phenolic content with phytochemical analysis were determined.

METHODS AND MATERIALS:

Collection of plant materials (seeds):

B. racemosa and C. fistula seeds were collected from different places in the Jodhpur, Jaisalmer, and Barmer districts of Rajasthan. The seeds were cleaned with water, air dried in shade for few days and finally crushed and grounded with mortar.

Extraction of seed oil:

The B. racemosa and C. fistula seed oils were extracted using a soxhlet apparatus and n-hexane (40^0-60^0C) was used as a solvent. For about 1 hour, the seed oils were refluxed with methanolic NaOH. Diethyl ether was used for extraction after the aqueous phase was acidified with hydrochloric acid. 2% H_2SO_4methanolic solution was added after the organic phase had been dissolved in hexane, and the mixture was refluxed for an hour. FAMEs were obtained after extraction and solvent removal.[15] The oils were analyzed for moisture content and nitrogen content by using standard methods.

Analysis of fatty acids composition:

Shimadzu with QP2010S type GC-MS spectrophotometer was used to assess the fatty acid content of seed oils. A capillary column made of Rtx-5MS column (BPX 70 TM; length: 30 m; diameter: 0.25 µm; film thickness: 0.25 µm) was utilized. The carrier gas was helium, with a flow velocity of 1 ml/min. The injector temperature was 240^0C, while the detector temperature was 250^0C. The oven temperature was set to 60^0C and subsequently increased to 150^0C at a speed of 6^0C/min for 15 minutes before reaching 200^0C at a pace of 8^0C/min for 15 minutes. For comparison, FAMEs with retention indices were employed. The measurements were taken thrice, and the average result was utilized to calculate the findings.

Analysis of Antioxidant activity:

The antioxidant activities of B. racemosa and C. fistula were evaluated by two methods:

Ferric reducing antioxidant power (FRAP)- The FRAP test was carried out in the manner reported earlier by Benzie and Strain.[16] The FRAP reagent was made by combining a 10:1:1 solution of acetate buffer, TPTZ, and FeCl3. An aliquot of the FAMEs (100 μ L) and 200 μL of distilled water was added to 2mL of the FRAP reagent and incubated at 35°C for half an hour in the dark. The absorbance was measured using a spectrophotometer at 593 nm, against a blank experiment. The results were contrasted with the standard curve that was meticulously crafted using varying amounts of Trolox.

The DPPH free radical scavenging activity was calculated using Cheung and coworkers'technique.[17]In brief, each extract received 0.2 mM DPPH at methanol (DPPH reagent) in a volume ratio of 4:1. A spectrophotometer was used to measure the absorbance at 520 nm after 10 minutes of exposure to low-light conditions. The standard is ascorbic acid. The antioxidant activity (AOA) was measured by the formula given below:-

$$\text{DPPH free radical scavenging activity} = \frac{\text{Abs of control- Abs of sample}}{\text{Abs of control}} \times 100$$

The data were presented as IC50 (inhibitor concentration)values, which are defined as the sample concentration that reduces the absorbance of the DPPH reagent by 50%.All the antioxidant activity tests were carried out in triplicate and data was expressed by mean ± S.D.

Analysis of Total Phenolic Content- Total phenolic content was determined using Folin-reagent, Ciocalteu's reagent.[18] In brief, 500 µL of each FAMEs was combined with Folin-reagent, Ciocalteu's then 1.5 mL of sodium carbonate was added, followed by 2.75 mL of dH2O, and the resulting solutions were centrifuged for 5 minutes.

The supernatants' absorbance was measured at a wavelength of 725 nm. Gallic acid equivalents (GAE) per gram of free amino acids (FAMEs) was the unit of measurement for total phenolic content. All evaluations were performed thrice, and data were reported as mean ±S.D Extraction of seed oil.

RESULTS AND DISCUSSIONS:

The seeds of B. racemosa and C. fistula were dark brown and reddish-brown in colour respectively and evaluated for physical properties. The physico-chemical properties of seeds are given in Table-1. The oil and protein content of seeds is high. Oil extracted (yield; 27 %w/w) from B. racemosa seeds and (yield; 44 %w/w) from C. fistula seeds and free from sediments. These seedscontain 19% and 21% protein respectively. The iodine value of B. racimosa and C. fistula seeds oil is 73 and 69 respectively. Saponification values of 125.6 and 105 for the seed oils under study are statistically significant; a high saponification value for the seed oils indicates the presence of fatty acids with a high molecular weight and a low amount of contaminants. The refractive index value of B. racimosa and C. fistulaseed oils were found to be 1.425 and 1.413 respectively. The oil contains both saturated (30 percent and 27.4 percent) and unsaturated (68.5 percent and 71.4 percent) fatty acids, according to the GC (figure-1 and 2) and GCMS (Table-2) data. The main acids present in the oil in B. racimosa and C. fistula seeds oil, were Myristic acid (1.3% and 0.8%), Palmitic acid (17.5% and 19.3%), Stearic acid (11.2% and 7.3%), Oleic acid (11.8% and 18.1%), Linoleic acid (55.3% and 52.2%) and Linolenic acid (1.4% and 1.1%).

Table 1 Physicochemical Properties of Seed Oil

S. No.	Properties	B. racimosa	C. fistula
1.	Oil %	27	44
2.	Protein %	19	21
3.	Moisture %	4.63	6.86
4.	Saponification Value	125.6	105
5.	Iodine Value	73	69
6.	Refractive Index	1.425	1.413

Table 2 Component fatty acid (uncorrected weight percentage) of seed oils determined by GC-MS

S. No.	Properties	B. racimosa	C. fistula
1.	Myristic acid	1.3	0.8
2.	Palmitic acid	17.5	19.3
3.	Stearic acid	11.2	7.3
4.	Oleic acid	11.8	18.1
5.	Linoleic acid	55.3	52.2
6.	Linolenic acid	1.4	1.1
7.	Others	1.5	1.2

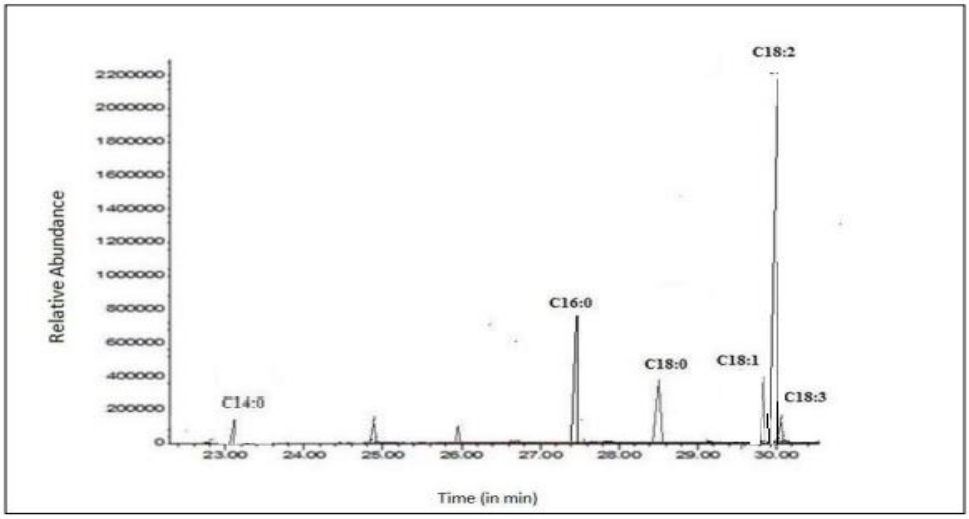

Figure 1 - GC-MS spectra of B. racimosaFAMEs

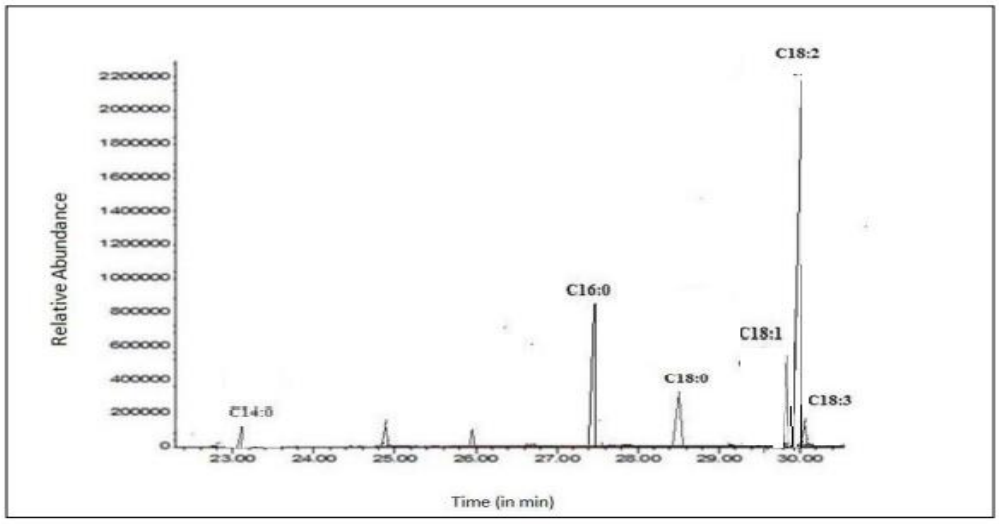

Figure 2 - GC-MS spectra of C. fistula FAMEs

Table 3 Total Saturated and unsaturated, MUFA and PUFA in B. racimosa and C. fistula seed oils

S. No.	Properties	B. racimosa	C. fistula
1.	Total saturated Fatty acid	30.0	27.4
2.	Total unsaturated Fatty acid	68.5	71.4
3.	MUFA (Oleic acid)	11.8	18.1
4.	PUFA (Linoleic acid + Linolenic acid)	56.7	53.3
5.	Others	1.5	1.2

Fatty acids in both B. racimosa and C. fistula mostly consist of linoleic acid. The only source of linoleic acid, an important amino acid, for humans is diet. In addition to protecting the human cardiovascular system, it aids

hypertension individuals in lowering their blood pressure [19, 20]. Some of its many uses include making soaps, emulsifiers, and fast drying oils, in addition to margarine, shortening, salad and culinary oils [21]. Oleic acid, the second primary chemical component, ranges from 11.8% to 18.1%. Of all the fatty acids found in human adipose tissue, oleic acid is by far the most prevalent [22]. Oleic acid has the potential to slow the development of the deadly brain and adrenal gland disorder adrenoleuko dystrophy (ALD) [23]. Olive oil's hypotensive effects are attributed to oleic acid as well [24]. Oleic acid is a pharmaceutical excipient that helps aerosol products dissolve or form emulsions [25]. Palmitic acid made up 17.5% and 19.3% of the oil from B. racimosa and C. fistula seeds, respectively, while stearic acid made up 11.2% and 7.3%. Soaps, candles, cosmetics, additives for food, lubricants, and non-drying oils are all made using palmitic acid (surface coatings). Alternatively, these seed oils may find usage in medicinal preparations, nutritional supplements, oil pastels, soaps, food packaging, deodorant sticks, toothpaste, and softening rubber due to the presence of stearic acid [26, 27].

In terms of antioxidant activity, both species' FAMEs interacted with and inhibited radical scavenging shown in Table-4. FAMEs derived from B. racemosa had stronger antioxidant activity than FAMEs obtained from C. fistula at all concentrations. FAMEs derived from B. racemosa had the higher DPPH scavenging activity of the two studied FAMEs. Further the B. racemosa FAMEs also had a lower value of IC_{50} (12.14±1.16 mg/ml). A lower IC_{50} value favors a higher antioxidant potency. [28] The radical scavenging activity of B. racemosa FAMEs was shown to be higher than that of C. fistula, which plays a key role in human diets as a health-promoting agent.

Table 4 Antioxidant activity By DPPH method of B. racimosa and C. fistula

Con. mg/ml	Ascorbic acid	B. racimosa oil	C. fistula oil
0.25	55.74±1.19	20.44±1.25	18.46±1.35
0.5	64±1.23	25±1.14	19±1.18
0.75	75±0.45	39±0.85	28±1.22
1.0	89±1.32	57±1.28	48±1.49
2.0	92±1.12	64±1.47	56±1.37
4.0	98±1.14	72±1.18	60±1.26

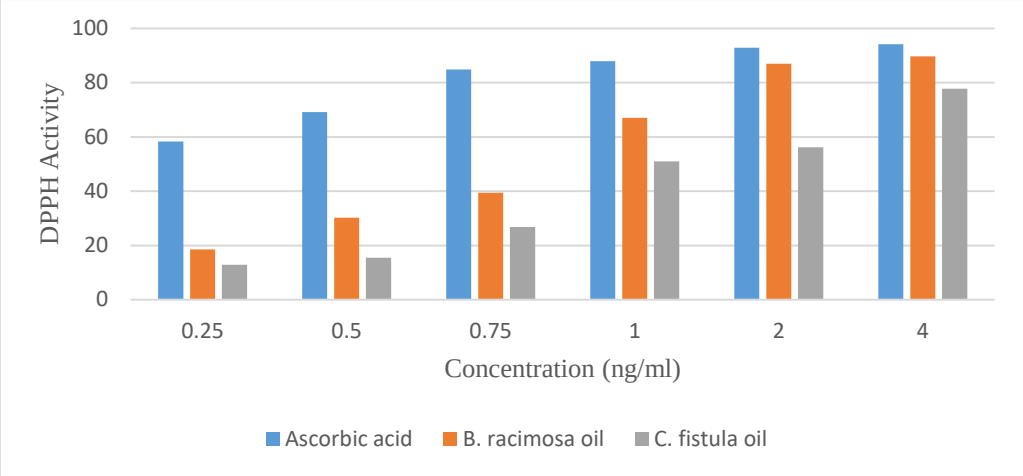

Figure 3- DPPH free radical scavenging activity of B. racimosa and C. fistula and ascorbic acid

The FRAP antioxidant activity reported for the FAMEs of B. racimosa and C. fistula were up to 89.73±1.38 μmolTE and 77.81±1.86 μmolTE (Table 5). Both DPPH and FRAP methods indicated that FAMEs of both the species have a higher antioxidant capacity, probably due to having a higher total phenolic content. The antioxidant activity analyzed for these species was higher than that shown by the apricot, peach, cherry, black cherry, and plum seed oils reported by Fratianni F, et.al. [29] The FRAP analyses confirmed the significant antioxidant potential of these species.

Table 5 Antioxidant activity By FRAP method of B. racimosa and C. fistula

Con. mg/ml	Ascorbic acid	B. racimosa oil	C. fistula oil
0.25	58.34±1.18	18.48±1.22	12.84±1.32
0.5	69.13±1.32	30.21±1.34	15.47±1.28
0.75	84.83±0.65	39.38±1.85	26.8±1.36
1.0	87.88±1.46	67.03±1.26	51.03±1.69
2.0	92.83±1.72	86.99±1.67	56.17±1.47
4.0	94.15±1.28	89.73±1.38	77.81±1.86

In terms of phenolic chemicals, the quantities detected in B. racimosa FAMEs (21.62±1.7mg EAG/kg) and C. fistula (18.86±1.8mg EAG/kg) (Table 6) are equivalent to those found in other vegetable oils.[30,31] Due to their potential health benefits, phenolic substances have sparked great research. Antiviral, anti-allergic, antiplatelet, anti-inflammatory, anticancer, and antioxidant properties have been found for phenolic content. [32] Gallic acid levels in both the species FAMEs were much higher than those discovered in walnut (3.39mg/100 g) by Donno.

D et al. [33] As previously noted, phenolic substances such as gallic acid have powerful antioxidant, antidiabetic, and antihyperlipidemic properties. [34]

Table 6 Total Phenolic Content of B. racimosa and C. fistula FAMEs and Gallic acid

Plant Species	Total phenolic content (mg GAE/g oil)
B. racimosa	22.46±1.8
C. fistula	19.82±1.2
Gallic acid	78.56±1.4

Based on the findings of this preliminary research, it is clear that the seed oil is nutritionally beneficial and has a high concentration of linoleic acid and oleic acid. It was discovered that B. racimosa and C. fistula might be a great way to get linoleic and oleic acid-rich natural oil. This study has the potential to provide light on potential uses for the oil and seeds of B. racimosa and C. fistula. These seed oils have been suggested as new sources of natural additives for pharmaceutical industries, since this study supports their antioxidant capability. There were nutritional and therapeutic benefits to the plant oils that were extracted and studied. The findings are equivalent to those of the conventional compounds, such as ascorbic acid, and they are compared to earlier results.

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