Comparative Phytochemical and Antioxidant Activities of Methanol and Petroleum Ether Extract of *Carissa Carandas* Leaves, Fruit and Seed

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Abstract

Carissa congesta Wight (syn. *Carissa carandas* Auct., previously it was known as *C. carandas* L.) carrying immense phytochemicals in its plant parts, is ethno pharmacologically highly significant for application in Ayurvedic, Unani, and Homoeopathic system of medicine. Owing to high medicinal importance, different plant parts are used for treatment of various health complications. Karonda fruits are frequently administered as astringent, and antiscorbutic. It is also applicable in curing anemia and biliousness. Its leaf puree is traditionally used in the treatment of fever, diarrhea, and ear ache, while the roots are very effective insect repellent, vermifuge and stomachic agent. The roots are also choice of remedy for itches. Studies on comparative evaluation of antioxidant potential of different plant parts are scanty. In light of available information, present research work is aimed to compare the free radical scavenging capabilities of petroleum ether and methanolic extracts of seed, fruit and leaves. Various parts of Carissa carandas, collected from RK University campus, were treated, dried and grounded to powder form which was then subjected to extraction by soxhlet apparatus. The Phytochemical analysis of extracts from different parts of C. carandas was performed followed by free radical scavenging ability using FRAP method. Results of reducing power ability of different extracts were compared with respect to each other through statistical analysis applying one-way ANOVA. Exploratory screening of phytochemical contents in petroleum ether and methanolic extracts of C. carandas leaves, fruit and seed extract demonstrated ubiquity of components including alkaloids, phenolics, flavonoids, oil and fats, saponins and tannins. Statistical analysis showed that methanolic extract of leaves was strong free radical scavenger among all other extracts under study. Results showed that the qualitative phytochemical screening conducted on the petroleum ether and the methanolic extracts of *C. carandas* leaves, fruit and seed extract confirmed the presence of some bioactive compounds (alkaloids, phenolic compounds, flavonoids, tannins, saponins, oil and fats) in its different parts. The methanolic extract of *Carissa carandas* leaves had highest free radical scavenging activity compared to petroleum ether extract of fruit and seed. The radical scavenging potential of the plants compared highly with that of standards, ascorbic acid. Results showed that the qualitative phytochemical screening conducted on the petroleum ether and the methanolic extracts of *C. carandas* leaves, fruit and seed extract confirmed the presence of some bioactive compounds (alkaloids, phenolic compounds, flavonoids, tannins, saponins, oil and fats) in its different parts. The methanolic extract of *Carissa carandas* leaves had highest free radical scavenging activity compared to petroleum ether extract of fruit and seed. The radical scavenging potential of the plants compared highly with that of standards, ascorbic acid. In conclusion, the methanolic extract of *C. carandas* methanolic leaves extract is more potent antioxidant and hence can be applied for various medicinal applications.

Keywords- *Carissa carandas*, Ethnopharmacology, Antioxidant, Phytochemicals, FRAP, Soxhlet.

Introduction

Carissa congesta Wight (syn. *Carissa carandas* Auct., earlier identified as *C. carandas* L.), is from Apocynaceae family (Jain S.K, 1991), widely spreaded throughout the Indian subcontinent and South East Asia. The shrub is more often referred as karonda (Devanagari), karamardaka (Sanskrit), Bengal currant or Christ's thorn (South India), Koromcha (Bengali), vakkay (Telugu), Karjatenga (Asam) and kilaakkaai (Tamil). Carissa carandas possess immense diversity of phytochemicals in its plant parts includes roots, stems, leaves and fruits making it ethanopharmacologically significant for application in Ayurvedic, Unani, and Homoeopathic system of medicine thus imparting huge pharmaceutical importance to the plant. Karonda has been center of research now a day because of its pharmacological significance. Research groups are working on investigation of the active components of it by using in-vitro and in-vivo approaches. Ascribed to its medicinal values, whole plant and its parts were used in remedial prescription to various ailments (Singh and Uppal, 2015). Fruits of karonda are the rich provider of vitamin C and iron when consumed in ample amount. Due to this, it is a common home remedy for treatment of anemia. Karonda fruits are frequently administered as astringent, and antiscorbutic. It is also applicable in curing anemia and biliousness. Its leaf puree is applied for treatment of fever, diarrhea, and ear ache, while the roots are very effective insect repellent, vermifuge and stomachic agent, the roots are considered as choice of remedy for itches. (Malik *et al.*, 2010).

The present study has been conducted to compare the antioxidant property of petroleum ether extract and methanolic extract of fruit, seed and leaves.

Materials and Methods

Plant Material collection and processing

Carissa carandas plant parts (leaves, fruits and seeds) were collected from RK University campus in January 2017 and were identified by Dr. Vivek Vegda (Asst. Professor, Botany, RK University, Rajkot). Plant parts (leaves, seed and fruit) were surface sterilized using 0.1% mercuric chloride and oven dried after three times washing with phosphate buffer saline. Dried samples were grounded into powder and preserved in airtight container.

Phytochemical extraction

In order to obtain crude phytochemical constituents, 25grams of dried plant part was subjected to extraction using 250mL solvents. Leaves extract was obtained using methanol (CLME) and petroleum ether (CLPE) while fruits and seed were subjected to petroleum ether extraction (CFPE and CSPE respectively). Extracts obtained after Soxhlet extraction were subjected to rotavapour to dried and stored in airtight container in refrigerator at 0-4°C for phytochemical analysis (Bint-e-sadek *et al.*, 2013).

Phytochemical screening

Phytochemical screening of the crude *Carissa carandas* plant parts (CLME, CLPE, CFME, CFPE and CSPE) were performed using standard protocols (Anupama *et al*., 2014) to ascertain the presence of alkaloids, carbohydrates, flavonoids, reducing sugars, phenolic compounds, protein, glycosides, tannins, saponins, oil and fats (Saxena and Pratap, 2017; Verma *et al.*,2015; Harborne, 1998).

In vitro antioxidant activity using FRAP method

Antioxidant activity of different extracts were compared using FRAP method as described by M. Oyaizu

(1986) with some minor variations. 500 µL of different extracts were used for one trial. In brief, from 5 mg/mL concentrated stock solution, 500 µL of extract was taken in separate test tubes. To this, 2.5 mL phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide (1%) was added and the mixture was incubated at 50^0C for 20 min. This was followed by adding equal volume of trichloroacetic acid (10%) to the previous solution. The mixture centrifuged for 10 min at 3000 rpm. From this, 2.5 mL supernatant was withdrawn in separate test tube to which equal volume of distilled water and 500µL ferric chloride solution (0.1%) was added. For the estimation of free radicals, absorbance of each extract was recorded at 700nm using UV–Visible spectrophotometer. Ascorbic acid was kept as the positive control. The experiment was repeated thrice.

Statistical Analysis

The comparative efficacy of different plant extract was determined statistically by applying one-way ANOVA and comparing the data using Tukey's post analysis. GraphPad Prism (v 5.1) was used to perform the statistical analysis.

Results and Discussion

Percentage of yield extract

After drying of 100 grams of fresh leaves of *C. carandas*, the methanol was used in phytochemical extraction process, and the percentage yield was estimated with respect to fresh mass and dry weight. The yield of methanol and petroleum ether seed, fruit and leaves extract of C.carandaswere14.4%, 11.28%, 5.7% and 24.74% respectively as mentioned in table 1.

Table 1: Extraction of phytochemical by Soxhlet method

Plant part	Solvent	Colour	Yield (per grams dry weight)	Yield (in percentage)
Seed	Petroleum ether	Yellow	0.85	14.4
Fruit	Petroleum ether	Colourless	0.88	11.28
Leaves	Petroleum ether Methanol	Green Green	0.94 0.75	5.7 24.74

Phytochemical analysis

Phytochemical screening analysis of petroleum ether and methanolic extracts of *C. carandas* leaves and petroleum ether extract of fruit clearly indicate the presence of different types of phytoconstituents (table 2). The results we obtained complies to previous reports, in which alkaloids, flavonoids, saponins, cardiac glycosides, triterpenoids, phenolic compounds and tannins and steroids, flavonoids, tannins, phenolic compounds, terpenoids, saponins, alkaloids and glycosides were found to be major phytochemical constituent (Sharma *et al*., 2007; Begum *et al*., 2013). The availability of phenolic and flavonoids in ample amount enhances the antioxidant activity of the extract, as these compounds are

widely reported to have antioxidant properties.

Table 2: Qualitative analysis of various phytochemicals in the plant extracts

S.No	Phytochemical tests		CLME	CSPE	CLPE	CFPE
1	Alkaloids	Mayer's test Wagner 's test Dragendroff 's test Hager's test	+	+	+	-
2	Carbohydrates	Molisch's test Benedict's test Fehling's test	+	+	+	+
3	Saponins	Froth test Foam test	+	-	+	+
4	Phytosterols	Salkowski's test	+	-	+	+
5	Tannins and Phenolic compounds	5% ferric chloride test Lead acetate test	+	-	+	+
6	Protein and amino acid test	Biuret test	+	+	+	+
7	Oils and fat	Spot test	+	+	+	+

Comparative in vitro antioxidant potential

In complex systems, various metabolic and environmental factors contribute to oxidative processes leading to genesis of different reactive oxygen and reactive nitrogen species from various target structures such as carbohydrates, proteins and lipids. These free radicals lead to cell and tissue damage. Plants being natural source of antioxidants have great potential to scavenge these free radicals. Thus, it is necessary to characterize the free radical scavenging activity of the plant extracts. The FRAP assay provides information on the activity of test compound/ extract with a suitable free radical. Ascorbic acid is taken as standard. The reducing power ability of fruit, seed and leaves extract was compared and results are summarized in table 3 and fig. 1. Comparative evaluation and its statistical analysis indicate that methanolic extract of leaves of karonda is having highest antioxidant ability compared to the fruit and the seed extract. The antioxidant activity and free radical scavenging found in *C. carandas* may be attributed to phytochemical compounds like flavonoids, phenols, saponins, alkaloids and tannins. Leaves of plant were having good percentage reduction values. From the results it can be concluded that the plant can be used as potent antioxidants for treating damages caused due to free radical generation in cells and resulting diseases.

Table 3: Reducing power ability of plant extracts as estimated by FRAP method

Plante xtract	Reducing power ability (AEAC µg/mL)
CSPE	64.58 ±2.7[a]
CFPE	24.11±3.72[b]
CLPE	24.97±2.32[b]
CLME	452.76±5.67[c]

All values are represented as mean± standard deviation.
Statistical analysis: One way ANOVA with Tukey's post-hock multiple comparison (alpha= 0.05, P value <0.05 is significant; n= 3)

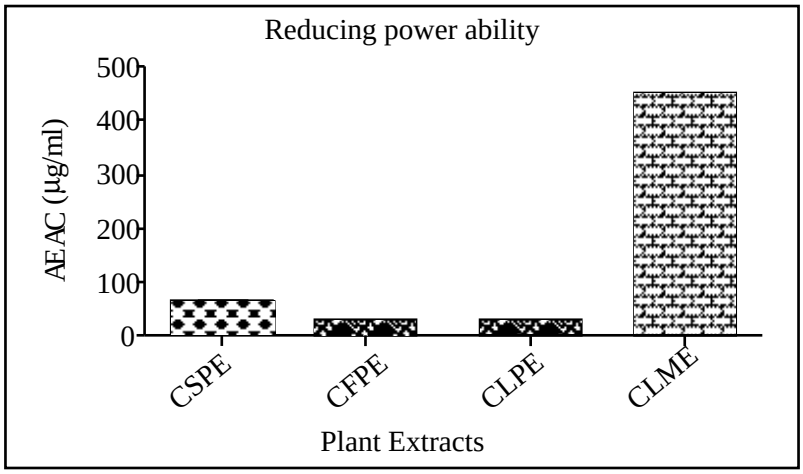

Figure 1: Reducing power ability of plant extracts as estimated by FRAP method

Conclusion

The present study revealed the ethnopharmacological properties of *C. carandas* plant parts (leaves, fruits and seeds) and provided immense proves for its medicinal properties due to presence of active phytochemicals. The antioxidant property of this plant may play a role in treatment of various diseases like diabetes, hepatic diseases, cancer and other metabolic disorders. Findings of this study take us a step ahead in the drug development process and new validated treatment of this traditionally used medicinal plant.

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