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Short Communication

Comparative analgesic activity of the root bark, stem bark, leaves, fruits and seeds of *Carissa edulis* VAHL (Apocynaceae)

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The analgesic activity of the water extracts (50,100 and150 mg/Kg body weight) of the root bark, stem bark, leaves, fruits and seeds of *Carissa edulis* were evaluated in mice using the mechanical method (tail-chip method) and chemical method (acetic acid induced writhing). The plant was found to have analgesic activity, with the fruits having the highest activity, followed by the leaves, seeds, root bark and stem bark respectively using metamizol as standard with mechanical method. There was slight variation with chemical method, the seeds were found to be most effective followed by fruits, leaves, root bark and stem bark respectively using acetylsalicylic acid as standard. The analgesic activity compared well with metamizol and aspirin which were used as standard, the chromatographic analysis indicate the presences of salicylates. These result justified the use of the plant in the treatment of toothache, lumbago, oedema and chest complaints by the traditional medical practitioners.

Key words: *Carissa edulis*, analgesic activity, metamizol, acetylsalicylic acid.

INTRODUCTION

Carissa edulis Vahl. (Apocynaceae) grows in tropical African region and Arabia (Irvine, 1961). The plant is commonly known among the Hausa in Northern Nigeria as 'cizaki' (Gbile, 1980), in Malawi as 'Mpambala Myoloko' (Sofowora, 1986); and its parts are used in folk medicine for wide varieties of remedies, such as fever, sickle cell anaemia and hernia (Audu, 1992; Yako, 1994). The plant parts are reported to be used for treatment of oedema, tooth ache, cough, ulcer, warm infestation and it is also used as source of dye (Banker and Verma, 1987; Burkill, 1985; Irvine, 1961; Oliver, 1960; Omino and Kokwaro, 1993; Sofowora, 1986).

The chemical compositions of the plant have extensively been reported. The extract yield benzenoids, penylpropanoid, lignans, sesquiterpenes and cumarins (Achenbach, 1983; Bentley, 1984) steroids, terpenes, tannins, flavonoids and cardiac glycosides, (Ibrahim, 1997). The ethnomedical uses of the plant and the need to establish its biological activity have prompted our interest. The comparative analgesic properties of the various parts of *C. edulis* are aimed at justifying some of the ethno medical uses.

MATERIALS AND METHODS

Plant materials

Samples of root bark , stem bark, leaves, fruits and seeds of *C. edulis* were collected in July, 1994 from bushes of Jama'a, a place between Aviation compound and Ahmadu Bello University (ABU) Samaru, Zaria. The plant was identified on the field using descriptions given in the literature (Hutchinson and Dalziel, 1963; Irvine, 1961). The identity of the plant was confirmed at the Herbarium, Biological Sciences, Ahmadu Bello University, (A. B. U.) Zaria. The voucher specimen number was 900132.

Preparation of extracts

The different parts of the plant namely root bark, stem bark, lea-ves, fruits and seeds were removed from the whole plant and air-dried separately at room temperature for fourteen days. The dried parts were powdered and sieved using 20 mesh sieve. 50 g of each of the powdered parts was boiled using distilled water in 250 ml beaker on a water bath for 3 h, filtered and lyophilised. The extracts were used to prepare a stock solution of 20 mg/ml for each sample.

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Table 1. Analgesic activity of the root bark, leaves, fruit, stem-bark and seed water extracts of *Carissa edulis* by mechanical method.

Time(min)	0	15	30	45	60	75	P value
Control	4.7±0.4	5.1±0.8	5.0±1.0	4.8±0.7	4.9±1.0	4.3±0.5	
Roots (50 mg/kg)	4.2±0.5	6.6±0.2	6.8±0.2	7.6±2.2	6.7±1.0	6.3±0.1	0.001
Stem (50 mg/kg)	4.8±0.0	5.6±0.1	6.2±1.3	6.5±1.6	4.9±1.0	5.1±0.9	0.001
Leaves (50 mg/kg)	5.4±2.6	7.7±2.4	8.1±2.3	8.7±0.4	5.8±0.5	4.9±0.4	0.0001
Fruits (50 mg/kg)	4.9±1.0	6.3±0.9	8.3±1.3	9.2±0.9	6.6±0.7	6.1±1.1	0.0001
(100 mg/kg)	4.9±0.7	7.4±0.7	9.1±1.0	8.1±0.2	8.6±0.7	6.7±1.0	0.0001
(150 mg/kg)	4.9±1.3	7.2±1.3	10.3±0.9	8.1±0.7	6.8±0.8	5.3±0.7	0.0001
Seed (50 mg/kg)	4.6±0.5	5.3±0.7	7.6±0.6	8.4±0.2	7.8±0.9	6.5±1.0	0.0001
Metamizol (10 mg/kg)	4.2±0.5	5.6±0.9	7.5±1.4	7.1±1.4	5.8±0.3	5.2±1.1	
(20 mg/kg)	5.0±0.8	7.3±1.4	7.7±1.0	7.5±1.0	5.9±1.0	5.3±1.1	
(30 mg/kg)	4.4±1.1	6.3±0.4	7.0±0.2	8.9±0.7	7.0±0.5	6.5±0.5	

Animals

Male albino mice (weighing 20 – 31 g) were used. The animals were bred in an animal house, Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria. The animals were fed on grower's mash (Sander's Feeds Limited Kaduna) and water was provided *ad libitum*.

Analgesic activity

Two methods were employed to conduct this test; the mechanical (tail clip Turner and Hebborn, 1971) and the chemical (acetic acid induced writhing) methods as described by Sigmund et al. (1957), Turner and Hebborn (1971) and Ragusa et al. (1992). The pain threshold was measured on a scale when the mice struggle. For each animals used at anytime, three readings were taken and the average recorded. The number of writhings occurring between 5 and 15 min after the acetic acid injection was recorded. The animals were divided into seven groups of six mice each. The first group serve as control (0.2 ml distilled water) and the second, third and fourth groups metamizol (analgin®) at doses of 10, 20 and 30 mg/kg was used as standard for mechanical method. The fifth, sixth and seven groups were given water extracts at doses of 50, 100 and 150 mg/kg body weight intraperitoneally to the mice. Same grouping was adopted for chemical method but acetylsalicylic acid was used at (Aspirin®) 150 mg/kg body weight intraperitoneally to the mice as the standard.

Statistical analysis

A statistical analysis of the result was expressed as Mean ± SEM. The statistical analysis was carried out using the student t-test with level of significance $p \leq 0.05$.

Chromatographic analysis

Paper and thin layer chromatographic analysis (PC, TLC) using silica gel G with ferric chloride as detecting reagent was used. Various solvents systems were tried, ethylacetate-n-butanol-acetic acid-water (50:20:15:15) giving the best results (BP. 1980; Geissmann, 1962; Stahl, 1969). The unhydrolysed and hydrolysed water extracts were co-chromatographed with methyl salicylate and salicylic acid as standard.

RESULTS

The results obtained are given on Tables 1 and 2. The analgesic activities of both methods (mechanical and chemical) were similar. Using the mechanic method the fruit sample was found to be the most effective followed by leaves, seeds, root bark and stem bark respectively at $P < 0.05$ (Ibrahim, 1997). The activity was found to be dose dependent. The onset of action was fast within 15 min of administration and the peak was obtained at 45 min, after which the activity of the drug sample was seen to decline

With the chemical method, the seed water extract was found to be most effective followed by the fruits, leaves root bark and stem bark respectively. The activity was found to be dose dependent in all the samples except that of the seeds extracts (where the 100 mg/kg was less active than 50 mg/kg); this might be due to biological variation in the animals. The activity of the 100 mg extracts is comparable to 150 mg acetylsalicylic acid. Co-chromatographic analysis using PC and TLC with some salicylates gave same violet spots and R_f values with 1% ferric chloride reagent indicating the presence of salicylates Table 3 (Ibrahim, 1997).

DISCUSSION

The water extracts were used as this was the form usually employed by the traditional medical practitioners and patients. The two different methods used (mechanic and chemical) showed the water extracts at 50, 100 150 mg/kg doses to posses analgesic activity. The activity was found to be significant at $P < 0.001$ and $P < 0.0001$ (mechanical method) and $P < 0.037$ (chemical method). The analgesic activity compared well with the standards 10, 20, 30 mg/kg metamizol; in some instance even higher than that of 30 mg/kg metamizol (fruit water extracts 50, 100 and 150 mg/kg water extracts). This indicates that the fruit sample could be used even at 50 mg/kg to

Table 2. Analgesic activity of the root bark, leaves, fruit stem-bark and seed water extracts of *Carissa edulis* by chemical method.

Extracts	Dose (mg/kg)	Mean of writhing
RWE	50	14±0.12
	100	12±0.`7
	150	9±0.27
LWE	50	12±0.22
	100	9±0.11
	150	9±0.24
FWE	50	12±0.07
	100	9±0.53
	150	7±0.24
SWE	50	9±0.13
	100	10±0.23
	150	5±0.12
SBWE	50	14±0.17
	100	13±0.24
	150	10±0.47
Control	0.2 ml	23±0.15
Standard	150	10±0.36

P<0.037
RWE = Root Water Extract, LWE = Leaves Water Extract, FEW = Fruit water Extract, SWE = Seed Water Extract, SBWE = Stem bar Water Extract, Control = Normal saline and Standard = acetylsalicylic acid (Aspirin®).

Table 3. Chromatographic analysis of the fruits water extracts.

Sample	PC		TLC	
	hRf%	Colour	hRf%	Colour ($FeCl_3$)
Unhydrolysed Water extract	98.0	Purple	52.8	Violet
	95.1	Violet	73.6	Violet
			86.0	Violet
			94.3	Violet
Hydrolysed water extract	95.0	Violet	72.0	Violet
			56.0	
Salicylic acid (standard)	94.0	Violet	88.7	Violet
Methylsalicylate (standard)	97.1	purple	94.3	Violet

compliment metamizol.

Usually in traditional medicine the fruits are not separated from the seeds. The results have shown that both have activity with the activity slightly varying with the method of analysis. Therefore there is no need to remove the seeds. The salicylates are known to have antipyretic, analgesic and anti-inflammatory actions which might be one of the compounds responsible for the analgesic activity of the plant *C. edullis.*

CONCLUSION

The obtained results justifies the use of the plant as analgesic in the treatment of toothache, ulcer, chest pains, lumbago, hernia and sickle cell diseases by the traditional medical practitioners.

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