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Anti-diarrhoea and phytochemical evaluation of *Phoniex dactylifera L.* extracts

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ABSTRACT

The date palm (*Phoenix dactylifera L.*) is known to be one of the oldest cultivated trees in the world, claimed to have anti-diarrhoea activity traditionally. Phytochemical components of the plant material are dependable sources for the treatment of health problems. Extract from fruit were screened for bioactivity against *Salmonella spp and Shigella spp*. The results significantly show activity at various concentrations for the distilled water, methanol and petroleum ether extracts. The crude and chloroform extracts revealed insignificant activities. Phytochemical analysis on the plant extracts reveals the presence of tannins, steroids, flavonoids and alkaloids. However, physiochemical analysis was also carried for the various solvent extract.

Introduction

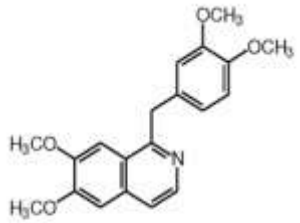
Plants are rich of secondary metabolite; such as tannins, alkaloids, flavonoids, phenols, steroids, glycosides, volatile oils, etc. (Cowan, 1999). It's important to identify the phytochemical and antimicrobial components of historical medicinal plants. Nowadays, most of drugs are derived from medicinal plants and as such it will be worthy important to screen and understand these plants used by our forefathers as medicines. In addition, investigations into antimicrobial activities of medicinal plants will expose the plant as potential source of therapeutic agents (Ebena et al., 1991). According to World Health Organisation (WHO), more than 80% of world's population relies on traditional medicine for primary health care needs.

The first generally accepted use of plants as healing agents was depicted in the cave pantry discovered in the Lascaux caves in France, which have been radiocarbon dated to be between 13,000-BCE. Anthropologist theorizes that overtime, and with trial and error, a small base of knowledge would have been acquired within every trial community. As this knowledge base expanded over the generation, the specialized role of the herbalist emerged (Rabe, 1997).

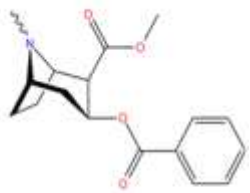
Higher plants, as source of therapeutic agents continue to play a great role in the maintenance of Human health since antiquities. Over 50% of all modern clinical drugs are of natural products origin. Thus, natural products play an important role in drug development programs of pharmaceutical industry (Suffiness and Duros, 1982; Baker *et al*., 1995; Cordell, 1995).

The use and search of drugs and dietary supplement derived from plants accelerated in the recent years. Pharmacologist, microbiologist, botanist, biochemist, and natural product chemists have teamed up to discover botanical drugs for treatment of various diseases, such drugs includes:

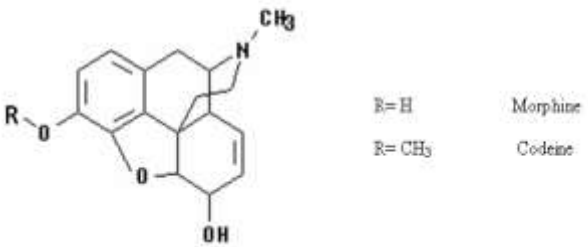
1. Papeverine: From Opium Poppy, used as smooth muscle relaxant (vasodilator)
2. Cocaine: Isolated from Erythoxylum Coca, which is use as a local anaesthetic and has been used in minor ear, nose and throat surgery.
3. Morphine and Codeine: From Opium Poppy use to kill pain (by relieving).
4. Griseofulvin: From a fungus; Penicillin Griseofulvin use as an antibiotic and antifungal.
5. Menthol: From peppermint use in nasal treatment.

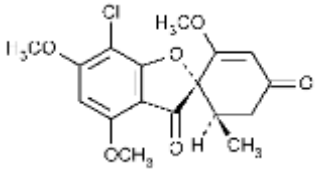


1. Paperverine



2. Cocaine

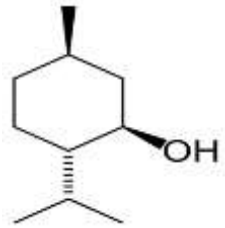




4. Griseofulvin

Tele:

E-mail addresses: mustcapture@yahoo.com



5. Menthol

Diarrhoea a Greek term meaning flowing through (Mediterms, 2012) is the condition of having frequent loose or liquid bowel movement. Diarrhoea is a common cause of death in developing countries and the second most common cause of infant death worldwide. A study released in Oct. 2009, suggest diarrhoea to be estimated to cause three times more death than previously thought at 1.1 million annualy for people aged 5 and over (WHO, 2005). According to UNICEF, diarrhoea kills up to about 1.5 million children with age under 5-years annually. Diarrhoea is given a first aid treatment by using oral dehydration solution (ORS), which is salt/sugar solution with modest amount of electrolytes and according to world health organisation (WHO) statistics, some 50 million children have been saved using a low cost treatment of oral rehydration salt. In the past 25 years (DuPont, 1997).

People of all ages can get diarrhoea. An average adult has a bout of acute diarrhoea about four times a year (DuPont, 1997). In United States America, a child will have had two episodes of diarrhoea 5 each year (Ramaswamy and Jacobson, 2001) at average young age. Most commonly causes of diarrhoea are bacterial infection, consumed through contaminated food; viral infections caused by viruses, parasite, functional bowel disorder, intestine diseases, food intolerance and sensitivities, and reaction to medicine such as Antibiotics.

The plant "Dabino" in Hausa Language and commonly known as Date palm, is a palm in the genus phoenix extensively cultivated for its edible sweet fruit. Due to its long history of cultivation for fruit, its exact native distribution is unknown, but probably originated somewhere in the desert oases of northern Africa, and perhaps also southwest Asia. It is an erect tree 30.5-36.5 m tall, often clumped with several trunks from a single root system with upward pointing, but often growing singly as well. The leaves are pinnate, up to 6 m long, with spines on the petiole and about 150 leaflets. The fruit is oblong, about 2.5- 3.5 cm long, it can be dark brown, reddish or yellowish-brown when ripe, the skin can be thin or thickish and the flesh is sweet until fully ripe. The fruit contain a hard seed inside. Full span of the crown ranges from 6-10 meters (Morton and Miami, 1987).

Date have high tannin content and are used medicinally as a detersive (having cleansing power) and astringent in intestinal trouble. It is also used as an infusion, decoction, syrup, or paste, dates may be administered for sore throat, cold, bronchial catarrh, and taken to relieve fever and number of other complaints. One traditional belief is that it can counteract alcohol intoxication. The seed powder is also used in some traditional medicines. A gum that exudes from the wounded trunk is employed in India for treating diarrhoea and genito-urinary ailments. The roots are used against toothache. The pollen yields an estrogenic principle, oestrone, and has a gonadotropic effect on young rats.

This paper reports a study on the antimicrobial activity of Date fruit against *Salmonella spp and Shigella spp*. Also, preliminary phytochemical analysis and physiochemical properties of various extracts of the fruit were similarly studied.

EXPERIMENTAL

Collection of Plant Material

Date fruits were obtained from local market in Kofar Wambai Kano Nigeria, which was believed to have been brought from Agades in Niger Republic as explained by the seller. The pits (seeds) were manually separated from the flesh. The flesh was then further dried for seven days and grounded to powder and stored in clean sample bottle under laboratory conditions prior to the extractions.

Extraction procedure

The method described by Yakasai, (2000) was adopted. About 500g of the air dried powdered plant was percolated with 600cm^3 of chloroform-ethanol (1:1) mixture for seven days. The extracts were drained, filtered and concentrated under reduced pressure using Rotary vapour (R 110, at 40^{o}C). The concentrated crude extract was then transferred into a clean evaporated beaker and allowed for complete evaporation under room condition. Weight of crude extract was determined and the fraction coded as MD01.

The crude extract MD01, was partitioned between chloroform (150 cm^3) and water (150 cm^3) in a separating funnel. The mixture was shaken for 15 minutes and allowed to stand overnight for proper separation between the two phases. The two phases were then carefully drained into separate beakers and coded as MD03 for the distilled water extract and MD02 for the chloroform extract. The MD03 layer was then evaporated in a vacuo, and the weight was determined. The product from the chloroform extract, MD02 was further partitioned between 90% methanol (150 cm^3) and petroleum ether 60-80 (150 cm^3). The mixture was shaken, allowed to stand for separation, separated, evaporated and weights were determine for each fraction of the methanol and petroleum ether and coded as MD04 and MD05 respectively.

Preliminary phytochemical analysis

The plant fractions, MD01, MD02, MD03, MD04, and MD05 were placed in five separate test tubes. Ethanol was added to each fractions and each mixture was then shaken vigorously and distributed into test tubes. The test for the presence of reducing sugar, tannins, steroid, flavonoids, alkaloids and saponins were carried out as follows.

Test for sugar

Additions of fehlings solution to the extract solution and warmed; bricks red precipitate signifies the presence of reducing sugar in accordance with Brain and Tunner (1975).

Test for tannins

About 2 drops of 5% $FeCl_3$ solution was added to the extract solution, a dirty green colour signifies presence of tannins as reported by Cluiei, (1994)

Test for alkaloids

Two drops of HCl were added to the extract and then followed by addition of few drops of Meyer's reagent. Alkaloid solution produces white yellowish precipitate (Sadiqqui and Ali, 1997). Most alkaloids are precipitated when in neutral or acidic solution by Meyer's reagent (Evans, 2002).

Test for steroids

Five drops of concentrated H_2SO4 were carefully added to 1 ml of the extract solution, red coloration indicates the presence of steroid (Salkowski test).

Test for flavonoids

About 4 mm of the extract solution was treated with 1.5 ml 50% methanol and then warmed. Magnesium powder was added, followed by 5-6 drops of concentrated HCl, a red

coloration showed the presence of flavonoids (Sadiqqui and Ali, 1997).

Test for saponnins

Appearance of bubbles after vigorously shaking the extract in a test tube indicates the presence of aponnins.

Antimicrobial secreening.

Preparation of sensitivity discs.

The paper disc techniques described by Bauer and Kirby (1971) was used in determination of the antimicrobial activities of each fraction. Disc of about 6.0mm diameter were prepared from Whatman No. 1 filter paper using a punch. Batches of the disc were dispersed in screw-capped bottles and sterilized by heating at about 160^{o}C for 1 hr in an auto clave.

Stock solution preparation

0.2 g of each extract was dissolve in 0.2 ml Di methyl sulphoxide (DMSO) to afford a stock of 10,000 ug/ml, another four different concentrations were prepared for each extract from the stock solutions, 5,000ug/ml, 2,500 ug/ml, 1,250ug/ml, and 625 ug/ml. 10 discs were introduced into each vial such that 0.1 ml of each concentration was transferred into the disc, to get them sock in the solution. The prepared discs were stored in the refrigerator until the subsequent day.

Agar plates preparations,

Nutrient agar (NA) was used to prepare the plates. NA is a selective medium for the isolation of bacteria; 28 grams of NA dispersed in a litre of distilled water and allowed soaking for 1 minute. It was then swelled to mix. The mixture was sterilized in an autoclave.

The mixture was poured into plate (petri dishes) and allowed to cool and solidify. The nutrient agar plates were dried in an oven to removed moisture. The petri dishes containing the nutrient agar were seeded with the test organisms by spread plate techniques (Muktar, 2006). Disc from different concentrations of the extract fraction were placed on the plate and incubated at 35^{o}C for 18-24 hr. before they were examined for zone of inhibition. The diameters of zone of inhibition growth were measured and express in millimetres (Muktar, 2006).

Results and discussion

Antimicrobial activity test of the five solvent extract obtained from the plant revealed that distilled water, methanol, and petroleum ether extracts showed significant activity against the Diarrhoea causing bacteria. Other solvent extracts (ethanol and chloroform extract) did not show significant zone of inhibition when compared with others (Tables 1, 2, 3, 4, and 5).

The biggest zone of inhibition and significant activity was obtained from the extract of methanol and distilled water at the extract concentration of 2,500 ug/ml, (Tables 3 and 4). Activity was also showed for the extract of petroleum ether but at a lower concentration of 1,250 ug/ml and 625 ug/ml and the inhibition zones are not as big as that of methanol and distil water extract but significant, (Table 5). The extract of ethanol and chloroform also showed activity but not significant as the zone of inhibitions are very small, (Tables 1 and 2).

The most attractive activities of the extract are significantly on the *Shigella* bacteria that showed considerable activity for the extract unless for the petroleum ether extracts that showed high activity for salmonella bacteria at very low concentration of 625 ug/ml (Table 5).

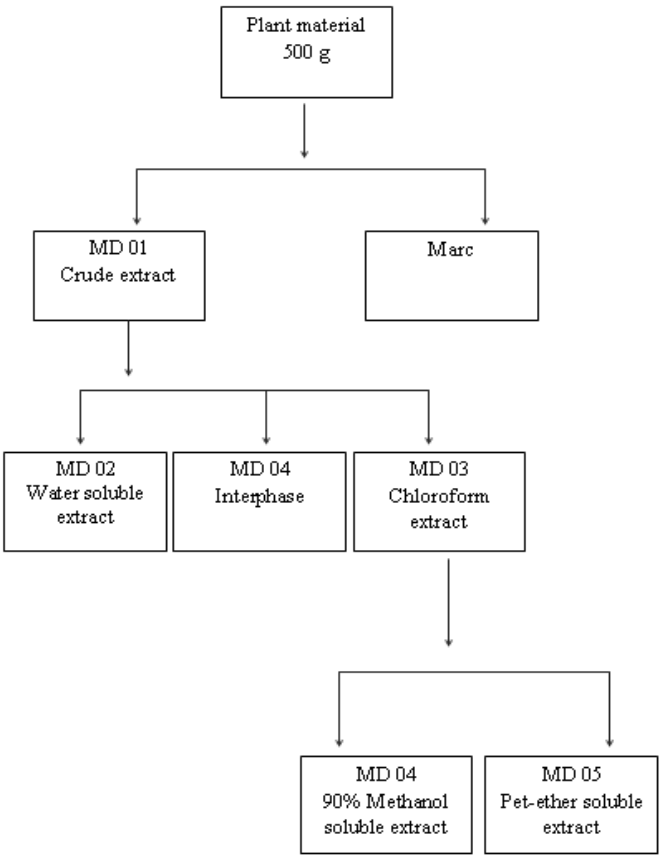
The analytical results for the analysis of phytochemical analysis of the plant extracts are given in the phytochemical screening table (Table 7). All the solvent extract revealed the presence of reducing sugar, tannins, steroids, alkaloids, flavonoids, and saponnins.

Physiochemical properties of the extracts (Table 6) showed the characteristics of each solvent extract and product yield from each extraction.

Activity of a plant material result typically from the component of the secondary metabolites present in the plant. This is usually attributed to the combination of these metabolites. The action of plant as medicine is unique to particular plant species or group, the distinct classification of the plant as a medicine depends particularly on the combination of the secondary compounds present in the plant (Parekh *et al*., 2005). Phytochemical screening of a plant is usually a part of the approach employed in choosing a plant for pharmacological study.

The plant extract give a study background for the antimicrobial potential of such a plant considering the different secondary compounds that are present in each partitioned extract, especially against bacteria causing diseases.

The results show the plant extracts of the date fruit to have good potentials in inhibiting bacteria growth but there effectiveness varied (i.e. from strong to weak). Plants extract antimicrobial activity has been reported to be classified as strong, medium, and/or weak (Zaika, 1998).



Scheme 1: scheme for the extraction and partitioning process

As antimicrobial plants will be a source for therapy of disease cause by microbes. The generic behaviour of such microbes provides them with the availability to resist action of the medicine. Thus, continual search for more potent medicine is recommended (Heisig, 2001).

There has been increased in the search and investigation of plant over decade as a source human disease therapy (Worldemicheal *et al*., 2003 Baek *et al*., 2004). This has been

attributed to many lapses on reports, and as a result not much valuable information is available for plants treatment of disease (Satish *et al*., 1999). This could also be due to the lack of information on the screening and evaluation of these plants on diseases causing bacteria.

In the present study, the plant, *Phoenix dactylifera L*. fruit was evaluated for antimicrobial potential against Diarrhoea causing bacteria, *Salmonella spp and Shigella spp*. The investigations clearly revealed the antimicrobial nature of the plant, and suggest the plant could be used in medicine preparation against these bacteria.

On the hand, phytochemical analysis reveals the presence of secondary metabolites which are the active ingredients of the medicinal plant. This provides an effective approach for the isolation of new medicinal active ingredient from higher plants (Duraipandiyan *et al*., 2006). Although, absence of certain secondary metabolites in one extract and its presence in the other extract may be attributed to various physiological and biosynthetic reactions taking place inside the plant (Farhat *et al*., 2001) and also effect of the environment should be considered as environmental activity may modify the activities of a plant.

Conclusion

The results of this study revealed that the extracts of *Phoenix dactylifera* contained pharmacologically active substance(s) with anti-diarrhoea properties. These properties may explain the rational for the effective use of the plant as an anti-diarrhoea agent in traditional medicine. However, further study is required to isolate and identify the individual active ingredients of the extract and study their precise mechanism of action. Castor oil induced diarrhoea transit test in rats for the extracts is also recommended to understand the actual action of the extract in humans.

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Tables 1-5: Results of antimicrobial activity

Zone diameter of inhibitions (mm) at various concentrations of the extract

Table 1: MD 01 (Crude extract)

S/No	Isolate	10.000 ug/ml	5,000 ug/ml	2,500 ug/ml	1,250 ug/ml	625 ug/ml
1	Shigella spp	08	09	11	14	10
2	Shigella spp	09	10	11	13	11
3	Salmonella spp	06	06	06	06	06
4	Salmonella spp	06	06	06	06	06
5	Salmonella spp	07	09	07	08	07

Table 1: MD 02 (Chloroform extract)

S/No	Isolate	10.000 ug/ml	5,000 ug/ml	2,500 ug/ml	1,250 ug/ml	625 ug/ml
1	Shigella spp	11	09	09	08	07
2	Shigella spp	09	08	09	09	09
3	Salmonella spp	11	08	06	06	07
4	Salmonella spp	10	07	08	08	09
5	Salmonella spp	09	08	09	09	08

Table 3: MD 03 (Distilled water extract)

S/No	Isolate	10.000 ug/ml	5,000 ug/ml	2,500 ug/ml	1,250 ug/ml	625 ug/ml
1	Shigella spp	25	16	34	28	06
2	Shigella spp	12	15	35	23	06
3	Salmonella spp	17	11	25	20	07
4	Salmonella spp	16	08	25	22	06
5	Salmonella spp	09	08	09	09	08

Table 4: MD 04 (Methanol extract)

S/No	Isolate	10.000 ug/ml	5,000 ug/ml	2,500 ug/ml	1,250 ug/ml	625 ug/ml
1	Shigella spp	08	06	40	17	13
2	Shigella spp	11	06	42	06	06
3	Salmonella spp	06	06	35	06	07
4	Salmonella spp	06	06	36	15	08
5	Salmonella spp	06	06	36	09	10

Table 5: MD 05 (Pet-ether extract)

S/No	Isolate	10.000 ug/ml	5,000 ug/ml	2,500 ug/ml	1,250 ug/ml	625 ug/ml
1	Shigella spp	06	06	06	16	18
2	Shigella spp	06	06	06	18	19
3	Salmonella spp	06	29	06	11	26
4	Salmonella spp	08	18	06	11	21
5	Salmonella spp	06	18	06	17	22

Key: - zone of inhibition less than 08 mm implies inactive (i.e. minimum inhibition concentration).

Table 6: Physiochemical properties of crude extract and partitioned fractions.

S/No	Extract fractions	Weight (g)	Colour / texture
1	MD 01	9.90	Light brown/ jelly-like solid
2	MD 02	1.50	Creamy/ gummy solid
3	MD 03	7.30	Dark brown/ gummy solid
4	MD 04	0.55	Pale yellow / waxy solid
5	MD 05	0.65	Dark brown / waxy solid

Table 7: Result of phytochemical analysis

2o metabolite group	MD 01	MD 02	MD 03	MD 04	MD 05
Reducing sugar	+ve	+ ve	+ ve	+ ve	-ve
Tannins	+ ve	+ ve	+ ve	+ ve	-ve
Steroids	+ ve	+ ve	- ve	- ve	+ ve
Flavonoids	+ ve	- ve	- ve	+ ve	-ve
Alkaloids	+ ve	+ ve	+ ve	+ ve	+ ve
Saponins	+ ve	+ ve	-ve	+ ve	+ ve