



RESEARCH PAPER

## GC-MS analysis of Phytoconstituents and Aphrodisiac Activity of the Root Extract of *Byrsocarpus coccineus* Schum and Thonn

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### ABSTRACT

*Byrsocarpus coccineus* Schum. and Thonn. (Connaraceae) (Short pod) is commonly found and widely used in many medicinal preparations for ailments in several parts of West and Central African countries. It is particularly used as stimulant for sexual drive in man. Previously, various parts of this plant were reported for treating different types of diseases, however there were no reports on GC-MS analysis of ethyl acetate fraction of this plant part. This study analyzed and identified the phytoconstituents of the ethyl acetate fraction of *Byrsocarpus coccineus* root extract using GC-MS analysis. The ground plant material was extracted with 70% methanol using maceration method. The crude extract was fractioned into soluble fractions. The preliminary phytochemical screening of the crude extract and ethyl acetate fraction were carried out according to standard procedures. The crude extract and the ethyl acetate fraction were screened for aphrodisiac activity. The extract yield was; crude 88.95%, *n*-Hexane 10.76%, ethyl acetate 25.00%, dichloromethane 20.40% and aqueous 36.40% respectively. The phytoconstituents present in both crude and ethyl acetate fraction include tannins, flavonoids, saponins, anthraquinones, steroids, cardiac glycosides and alkaloid respectively, except for phlobatannins that was absent in both. The crude and ethyl acetate fraction of *Byrsocarpus coccineus* root were subjected to aphrodisiac activity using wistar male albino rats. Both showed more activity at higher concentration (400 mg/kg body weight) on male wistar albino rats as evidenced by an increase in number of mounts mating performance. The activity of the ethyl acetate fraction was more in all the doses tested than the crude extract, which is an indication that the ethyl acetate fraction contains more concentrated of the phytoconstituents responsible for sexual drive. The ethyl acetate fraction been the most active fraction was subjected to GC-MS test for identification phytochemicals. The GC-MS analysis showed thirty (30) peaks of different phytoconstituents amongst them are: 2-cyclohexen-1-one, catechol, 2-amino imidazole-5-propionic acid, 4-methoxybenzene-1, 2-diol, hydrocoumarin, 2, 4-dimethoxyphenol and 2, 6-hydrobenzaldehyde. These phytochemicals may be responsible for the aphrodisiac activity of *Byrsocarpus coccineus* root extract. Some of these identified compounds were reported as having antimicrobial activities, which justifies the use of the root extract of *Byrsocarpus coccineus* for the treatment of various ailments by traditional practitioner. Therefore, the phytoconstituents present in the ethyl acetate fraction of *Byrsocarpus coccineus* root may be beneficial for patients suffering sexual disorders as proclaimed by the traditional healers.

**Keywords:** Phytochemicals, Aphrodisiac activity, Wistar albino rats, *Byrsocarpus coccineus*, GC-MS.

### INTRODUCTION

Plants have been known as the natural sources of medicinal agents against many diseases and as such it continued to play a dominant role in the local and primary health care of about 80% of the world's population (Ahmadu *et al.*, 2006). In Nigeria, more than 70% of the estimated 140 million people are rural dwellers, who depend largely on indigenous herbal medicines as source of their health care (Mann

*et al.*, 2003; Akindele and Adeyemi, 2007). Ethnomedicinal studies are very important in the provision of health delivery. Such studies helped in the identification of medicinal values

Received 03 March, 2017

Accepted 19 May, 2017

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of these plants which lie in the bioactive phytochemical constituents that produce definite physiological actions on the human body. These bioactive phytochemical constituents in medicinal plants include alkaloids, flavonoids, phenolic compounds, tannins and essential oils (Lawal *et al.*, 2010; Bashir *et al.*, 2015).

Good knowledge of phytochemical compounds is not only supportive for discovery of therapeutic potential, but also contribute actively towards discovery of new semi-synthetic and synthetic compounds (Ngo *et al.*, 2013). The novel molecules from plant sources have been instrumental in development of structurally modified compounds, which assist a lot in the development of modern therapeutic system (Newman and Cragg, 2007).

The screening of plant extracts is an innovative strategy to find therapeutically active compounds in many plant species. Hence, Gas chromatography and Mass spectroscopy (GC-MS) associated with particular detection techniques have become a sophisticated means for analysis of various compounds (Hostettmann *et al.*, 1996).

Sexual dysfunction, erectile dysfunction or male impotence is characterized by the inability to develop or maintain an erection of the penis and can be caused by psychological disorders like anxiety, stress and depression, physical disorders like chronic diseases: diabetes and hypertension; hormonal problems or sedentary life-style, alcohol and smoking abuses (Malviya *et al.*, 2011; Sumalatha *et al.*, 2010).

Some oral medications are available and well-established for sexual dysfunction treatment, among of them are the phytoconstituents with aphrodisiac property: cantharidin (Spanish fly) and yohimbine, quercetin, papaverine and berberine (Pallavi *et al.*, 2011; Patel *et al.*, 2011; Silva *et al.*, 2012; Semwal *et al.*, 2013). Aphrodisiac is the word derived from Aphrodite, the Greek goddess of sexual, love and beauty. An aphrodisiac is defined as an agent (food or drug) that arouses sexual desire. Current sexual dysfunction therapy lack satisfactory success due to adverse effect, hence patients are seeking complementary and alternative medicine to treat sexual dysfunction (Silva *et al.*, 2012).

Many Nigerian plants have been scientifically screened and found with significant aphrodisiac potentials in male wister rats (Singh *et al.*, 2013; Yakubu *et al.*, 2008; Yakubu and Akanji, 2011). Currently, two potential aphrodisiac compounds namely: xanthones (1,3,6,8-tetrahydroxy-2,5-dimethoxy xanthone and 1,6,8-trihydroxy-2,3,4,7-tetramethoxy xanthone) isolated from the root bark of *Securidaca longepedunculata* relaxed the corpus cavernosal smooth muscle by 97 % in comparison to sildenafil (Viagra) at  $1.8 \times 10^{-5}$  mg/ml (Meyer *et al.*, 2008). Aqueous extract of root of this plant has been reported to be safe when administered orally in mice (Etuk *et al.*, 2006). These purified phytochemicals may be picked up for large scale clinical trials in drug discovery programmes.

*Byrsocarpus coccineus* Schum and Thonn (Connaraceae) is commonly called huntsman's pepper or short pod. In Northern Nigeria, it is a popular plant with several local names: Darabagi (Nupe); Kimbar maharba or Tsamiyar-kasa (Hausa); Wangarabubi or Yangara-bubihi (Fulani); Kanti-kanti, amùjẹ wẹwẹ (Yoruba); Oke abolo or Mybo-apepea (Igbo) (Mann *et al.*, 2003).

*B. coccineus* is a shrub up to 2m high with light brown stem and very fine lenticel. Leaves are pinnate, petiole 1-2cm long with rachis and 6 pairs of oval glabrous (Plate 1). Fruits are capsular, glabrous, oblong and yellow about 15mm long by 7mm broad, which are slightly convex at one side, showing a black seed with orange aril. It is a shrub with prominent and numerous lenticules, pinnate leaves, 6-9 pairs of leaflets that are larger near rounded apex. It has small white or pinkish scented flowers usually between January to March (Burkill, 1985; Mann *et al.*, 2003).

*B. coccineus* has been known and used as sexual enhancers in Nupeland, as well as for the treatments of venereal diseases, dysentery, tumours, mouth and skin sores, sexual problems and impotence in several parts of West Africa (Mann *et al.*, 2003). The ethnomedicinal uses of this plant are supported by scientific reports of diverse range of biological activities including: analgesic, antidiarrhoeal, anti-inflammatory, antipyretic, antioxidant and antimicrobial activities (Amos *et al.*, 2002; Oke and Hamburger, 2002; Adeyemi *et al.*, 2010; Wazis *et al.*, 2013a; Atawodi, *et al.*, 2014).

These activities are due to the natural products isolated from various parts of this plant which includes: coumarins and its derivatives, quercetin 3-O- $\alpha$ -arabinoside (I), quercetin (II), quercetin 3- $\beta$ -D-glucoside, Stigma-5-en-O- $\beta$ -glucoside (Vickery and Vickery, 1980; Ahmadu *et al.*, 2007; Wazis *et al.*, 2013b). However, there is no report on the GC-MS analysis of the ethyl acetate fraction of *Byrsocarpus coccineus* root. Therefore, this study was aimed at isolating the phytochemical compounds responsible for the aphrodisiac activity in the ethyl acetate fraction of *Byrsocarpus coccineus* root.



Plate1: The leaves (A) and roots (B) of *Byrsocarpus coccineus*

## METHODOLOGY

The roots of *Byrsocarpus coccineus* were collected from a bush opposite the Central Gate of the Niger State Polytechnic, Zungeru, Niger State, Nigeria in August, 2014 and authenticated at the Herbarium section, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria; where a voucher specimen (864) was deposited.

### Preparation of crude methanol extract of the root of *B. coccineus*

The roots of *B. coccineus* collected were properly washed, chopped into pieces and air dried under laboratory conditions for two weeks. The dried root sample was pulverized to fine powder and extracted by percolation (thrice) in aqueous methanol (70% w/v) in large stoppered bottle at room temperature for 72 h. The crude root extract of *B. coccineus* was obtained by gravity filtration with Whatman No.1 filter paper. The solvent solution was concentrated *in vacuo* using rotary evaporator

(R E-600, Shendi, Shanghai) and it was further dried in a water bath and air dried to obtain the crude root extract (Mann *et al.*, 2012).

### Partitioning of the crude methanol extract of the roots of *B. coccineus*

The crude methanolic root extract of *B. coccineus* was dissolved in 70 % methanol (250 cm<sup>3</sup>) and transferred into 500 cm<sup>3</sup> separatory funnel. It was then partitioned with 250 cm<sup>3</sup> each of *n*-hexane dichloromethane, ethyl acetate and water to give *n*-hexane, dichloromethane, ethyl acetate and aqueous soluble fractions (Mann *et al.*, 2012). Each solvent soluble fraction was evaporated over a water bath and subsequently air dried and used for further analyses.

### Qualitative phytochemical analyses of crude methanol extract of the roots of *Byrsocarpus coccineus*

The crude extract was subjected to phytochemical analysis qualitatively for the presence of some secondary metabolites such as alkaloids, tannins, glycosides, steroids, terpenoids, flavonoids, saponins and resins, using standard methods (Mann *et al.*, 2012).

### GC-MS Analysis of the ethyl acetate partitioned fraction of *Byrsocarpus coccineus* root

The GC-MS analysis of the ethyl acetate partitioned fraction was performed using the GC-MS spectrometer (QP-2010; Shimadzu, India) which is a gas chromatograph interfaced to a Mass spectrometer equipped with elite-5MS (5% diphenyl /95% dimethyl polysiloxane), with a fused capillary column (30 x 0.25  $\mu$ m ID x 0.25  $\mu$ m df ). For GC-MS detector, an electron ionization system was operated in an electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1cm<sup>3</sup>/min, and an injection volume of 2  $\mu$ l was employed (a split ratio of 1:10). The injector temperature was maintained at 250°C, the ion source temperature was 200 °C, the oven temperature was programmed from 110 °C (isothermal for 2 min), with an increased temperature of 10 °C/min to 200 °C, then 5 °C/min to 280 °C, ending with 9min isothermal at 280 °C. Mass spectra were taken at 70 eV, a scan interval of 0.5s and fragments from 29 to 450 Da. The solvent delay was 0.0

to 2 min, and the total GC-MS running time was 46 min.

The relative percentage amount of each component was calculated by comparing its average peak area to the total peak area. And the mass-detector used in this analysis was Turbo-Mass Gold-Perkin- Elmer, and the software adopted handled mass spectra and chromatographs.

### GC-MS analysis and identification of phytoconstituents

The GC-MS analysis was carried out on the combined fraction F<sub>900</sub> at National Research Institute for Chemical Technology (NARICT) Zaria, Kaduna State, Nigeria, using GCMS model QP2010 PLUS (Shimadzu, Japan). Identification and interpretation of mass spectra GC-MS was carried out by comparing the mass spectrum of each compound with the mass spectral database of National Institute for Standard Technology NIST05.LIB Library (Ajayi *et al.*, 2011).

### Aphrodisiac activity Test

Healthy adult albino rats, weighing between 150-200 g were obtained from the animal house of the Department of Biological Sciences, Niger State Polytechnic, Zungeru. The animals were fed with standard pelleted diet and provided with water *ad libitum*. Aphrodisiac activity was done according to standard methods of Yakubu and Akanji (2011) and Wani *et al.* (2011) which were slightly modified as follows: the sexually active male rats were selected for testing aphrodisiac activity of the extract. The female rats were housed in a separate cage with food and water *ad libitum*. They were brought in oestrous phase by treating them with estradiol valerate (10 µg/kg and hydroxy progesterone 1 cm<sup>3</sup>/kg for 48 hours and 5 hours respectively, prior to experimentation in order to make them sexually acceptable. The sexually active male rats were chosen separately and divided into six (6) groups; each group consists of one male and two female rats. The male rat each groups received treatment orally. Four groups received the plant extracts (methanol crude extract and ethyl acetate fraction) in various concentrations (50 mg/kg, 100 mg/kg, 200 mg/kg, and 400 mg/kg), while the negative control group received only water and the positive control group was treated with commercially prepared

orthodox drug (Kedi Revive). After 30 mins of treatment, two female rats were introduced to each group. The sexual behaviors of the experimental animals were observed under dim light in a special designed cage with the aid of a digital camera. The mount frequency of the male rats in each group was recorded for 2 h in day1, day2 and day7 respectively (Wani *et al.*, 2011).

## RESULTS

### Qualitative phytochemical composition of methanolic root extract of *B. coccineus*

The qualitative phytochemical screening of the crude extract and the ethyl acetate fraction revealed the presence of tannins, flavonoids, saponins, anthroquinones, steroids, cardiac glycosides and alkaloids but phlobatannins and reducing sugar were absent in both extracts (Table 1).

**Table 1: Phytochemical screening of *Byrsocarpus coccineus***

Secondary metabolites EAF	Tests	CME	
Flavonoids	Shinoda test	+	+
	NaOH test	+	+
Tannins	Ferric chloride test	+	+
	Lead acetate test	+	+
Saponins	Froth test	+	+
	Kede test	+	+
Alkaloids	Mayers test	+	+
	Dradendroff's test	+	+
Steriods/tripterpenes	L/Burchard test	+	+
	Salkowski test	+	+
Cardiac glycoside	Keller-kiliani test	+	+
Anthraquinone	Borntragers test	+	+
	Combined test	+	+
Phlobatannins	HCl test	-	-
Reducing sugar	Molisch test	+	-
	Fehlings test	+	-

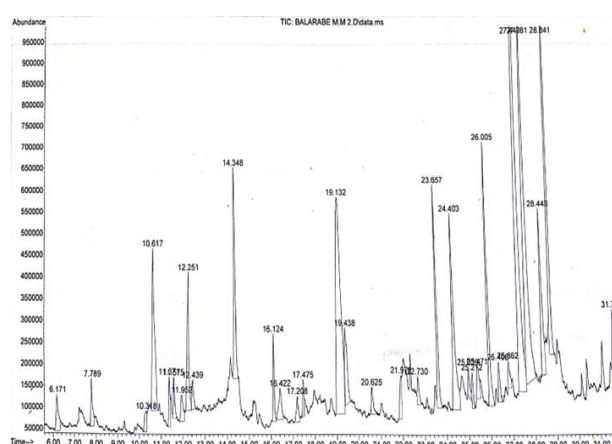
Keys: - = absent, + = present, CME = Crude extract, EAF = Ethyl acetate fraction

**Table2:** Effects of crude extract and ethyl acetate fraction of *Byrsocarpus coccineus* on male albino rats for three days after administration

Group	Concentration(mg/kg)		Mount		Frequencies		
	Crude extract		Ethyl acetate fraction				
	Day 1	Day 2	Day 7	Day 1	Day 2	Day 7	
A - control (water)	0.0	1.0	0.0	0.0	0.0	1.0	
B + control (Kedi Revive)	12.0	7.0	2.0	13.0	5.0	3.0	
C	50	0.0	1.0	0.0	0.0	2.0	1.0
D	100	1.0	1.0	0.0	2.0	2.0	1.0
E	200	5.0	1.0	1.0	5.0	2.0	1.0
F	400	9.0	5.0	2.0	10.0	6.0	2.0

**GC-MS identification of compounds**

The GC-MS spectral analysis of the ethyl acetate fraction *Byrsocarpus coccineus* extract revealed the presence of thirty (30) bioactive compounds. Amongst the identified compounds from the ethyl acetate fraction are 2-cyclohexen-1-one, catechol, 2-amino imidazole-5-propionic acid, 4-methoxybenzene-1,2-diol, hydrocoumarin, 2, 4-dimethoxyphenol and 2, 6-hydrobenzaldehyde. Some of these compounds may be responsible for the aphrodisiac activity of *Byrsocarpus coccineus* root extract. These compounds are presented on Table 3 according to their retention time, area %, fragmentation peaks, molecular formula and molecular weight.



**Figure I:** GC-MS chromatogram of ethyl acetate fraction of *Byrsocarpus coccineus* root extract

**Table 3:** Chemical compounds deduced from GC – MS spectrum of ethyl acetate fraction

Peaks	RT	Name of Compounds	Molecular formula	MW	Peak areas %
1	6.17	4-cyclopentane-1,3-dione	C <sub>5</sub> H <sub>4</sub> O <sub>2</sub>	96.08	0.72
2	7.79	Aziridine(1,1-dimethylethyl)	C <sub>6</sub> H <sub>13</sub> NO	117.19	0.66
3	10.32	Dimethoxy dimethyl	C <sub>6</sub> H <sub>10</sub> O <sub>2</sub> Si	134.21	0.35
4	10.62	Phenol	C <sub>6</sub> H <sub>5</sub> OH	94.00	3.19
5	11.37	Trolamine	C <sub>6</sub> H <sub>13</sub> NO <sub>3</sub>	149.19	0.54
6	11.58	2-cyclohexen-1-one	C <sub>11</sub> H <sub>14</sub> O <sub>2</sub>	178.23	0.46
7	11.95	N-butyric acid	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>	200.32	0.51
8	12.25	Pantolactone	C <sub>8</sub> H <sub>10</sub> O <sub>3</sub>	130.14	2.02
9	12.43	Tridecanone	C <sub>13</sub> H <sub>26</sub> O	198.34	0.40
10	14.35	2-methoxy (phenol)	C <sub>7</sub> H <sub>10</sub> O <sub>2</sub>	130.18	2.69
11	16.12	Benzyl nitrile	C <sub>8</sub> H <sub>9</sub> N	125.21	0.81
12	16.42	1,3-dioxolane	C <sub>2</sub> H <sub>4</sub> O <sub>4</sub>	412.65	0.68
13	17.21	1-trimethyl silyl	C <sub>16</sub> H <sub>26</sub> OSi	262.46	0.42
14	17.48	4-methyl silyloxyoctane	C <sub>13</sub> H <sub>29</sub> OSi	229.45	0.39
15	19.13	Catechol	C <sub>6</sub> H <sub>6</sub> O <sub>2</sub>	146.23	8.33
16	19.44	2-amino imidazole-5-	C <sub>4</sub> H <sub>4</sub> N <sub>2</sub> O <sub>2</sub>	172.20	1.43
17	20.63	4-methoxybenzene-1,2-diol	C <sub>8</sub> H <sub>10</sub> O <sub>3</sub>	176.25	0.43
18	21.98	3,7,7-trimethyl 8-bicyclo	C <sub>7</sub> H <sub>12</sub> O	234.42	0.67
19	22.73	2-phenylsulfonaminoethyl	C <sub>16</sub> H <sub>18</sub> N <sub>2</sub> O <sub>2</sub> S	314.49	0.39
20	23.66	Phenol (2,6-dimethoxy-)	C <sub>8</sub> H <sub>10</sub> O <sub>3</sub>	160.21	2.22
21	24.40	Hydrocoumarin	C <sub>9</sub> H <sub>8</sub> O <sub>2</sub>	154.21	3.48
22	25.04	3-pyridne carboxylic acid	C <sub>6</sub> H <sub>5</sub> NO <sub>4</sub>	183.16	0.38
23	25.21	Z-10-tetradecan-1-ol acetate	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	254.41	0.37
24	25.47	1-ethyl-2-hydroxy-2,3-dimethyl	C <sub>12</sub> H <sub>22</sub> O <sub>3</sub>	214.30	0.63
25	26.00	Coumarin	C <sub>9</sub> H <sub>6</sub> O <sub>2</sub>	152.19	2.90
26	26.41	2,4-dimethoxyphenol	C <sub>8</sub> H <sub>10</sub> O <sub>3</sub>	160.21	0.55
27	26.41	2,6-hydrobenzaldehyde	C <sub>8</sub> H <sub>8</sub> N <sub>2</sub> O <sub>3</sub>	201.22	0.54
28	27.44	Benzene acetonitrile	C <sub>7</sub> H <sub>7</sub> NO	125.17	10.43
29	27.78	1,1-dimthyl ethyl	C <sub>12</sub> H <sub>22</sub> O	188.35	7.91
30	28.45	3,4,5-trimethoxy phenol	C <sub>9</sub> H <sub>10</sub> O <sub>4</sub>	190.24	2.28

## DISCUSSION

The phytochemical test of the crude methanolic extract and the ethyl acetate fraction showed the presence of flavonoids, tannins, alkaloids, saponins, steroids, cardiac glycoside, anthroquinones, while both showed the absence of phlobatannins. Phytochemicals such as the terpenoids, steroids and saponins are responsible for the activities of the Central Nervous System and as such they tend to have analgesic and antidiabetic properties (Liu, 2003; Rupasinghe *et al.*, 2003). It has been found that more highly oxidized phenols are more inhibitory to microorganisms (Scalbert 1991). Flavonoid compounds inhibit multiple viruses. Many human physiological activities and wide range of anti-infective actions have assigned to tannins (Haslam 1996). Saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have anti-inflammatory effects (Liu, 2003; Akindele and Adeyemi, 2007). From the foregoing, the roots of *Brysocarpus coccineus* may have compounds that were similar to those ones with proven aphrodisiac activity (Silva *et al.*, 2012). However, a clinical trial conducted on the aphrodisiac activity of *Mucuna pruriens* where the powder was administrated at 5 gm/day orally once, showed significant improvement in sperm count and motility (). More so, flavonoids and other phenolic compounds; alkaloids, xanthines and other amines; and saponins were well reported for their aphrodisiac potency (Silva *et al.*, 2012); which are the classes of phytoconstituents found to be present in the ethyl acetate fraction of the root extract of *Brysocarpus coccineus*. The results of GC-MS analysis of the ethyl acetate fraction of *Brysocarpus coccineus* root extract revealed thirty (30) compounds as shown in Figure 1 above further confirmed this observation. Some of the identified compounds were earlier reported as having biological activities: antioxidant, antimicrobial and anti-inflammatory activities (Cowan, 1999). Specifically, phenolic compounds and others such as: catechol, 2-amino imidazole-5-propionic acid, 4-methoxybenzene-1, 2-diol, hydrocoumarin, 2, 4-dimethoxyphenol and 2, 6-hydrobenzaldehyde (Table 3) may be responsible for the aphrodisiac activity of *Brysocarpus coccineus* root extract.

## Conclusion

Correlating the phytoconstituents with the biological activities is on-going innovative research strategic thinking. *Byrsocarpus coccineus* is a plant, traditionally used for the treatment of venereal diseases, dysentery, mouth and skin sores and sexual problems. In this study, the root extract of *Byrsocarpus coccineus* have several therapeutic potentials and the phytoconstituents which are dose dependent can enhance overall sexual performance. Here in, we first time report the presence of some important compounds in this plant identified by GC-MS analysis. Thus, this type of study may give information on nature of active principles present in the medicinal plants. These identified phytoconstituents presumed to be responsible for traditional activity of *Byrsocarpus coccineus*.

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