PHYTOCHEMICAL ANALYSIS OF *CLEOME VISCOSA*.

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APPROVAL PAGE

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DEDICATION

I dedicate this research project to my late mother Hajiya Aisha Tukur Tambuwal.

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Thanks to Almighty Allah, the sole of the universe, may his blessing be upon his Prophet Muhammad (S.A.W) his companions and his family members .

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ABSTRACT

Cleome viscosa Linn (Capparaceae) commonly known as “wild or dog mustard” is an annual sticky herb found as a common weed all over the plains of India and through the trophics of the world. The whole plant and its parts (Leaves, pods and roots) are widely used in traditional and folkloric systems of medicine. Cleome viscosa were extracted with and all these extract were screened for the presence of various metabolites (primary and secondary) including proteins, carbohydrates, etc was found in the roots and the alkaloids, glycosides, amino acids, volatile oils, steroids and terpenoids are present in all three parts of C. viscosa, the result showed that.

CHAPTER ONE

1.0 INTRODUCTION

Definition

The *Cleome viscosa Linn* is commonly known as Asian spider flower or yellow spider flower. It belongs to Capperaceae family.*C.viscosa* is a weed distributed throughout the tropics of the world and the plains of india. It is known as Asian spider flower in English, Namijin `yaranguwa in Hausa, Hurhur in India, Hurhuria in Bengali, Nayikkadugan in Tamil (Asolkar*et, al.* 1992).Traditionally, this plant is used in various disorders such as diarrhoea, fever, inflammation, liver diseases, bronchitis, skin diseases, and malarial fever (Henty and Pritchard, 1975). The juice is useful in piles, lumbago and earache. The analgesic, antipyretic and anti-diarrhoeal activities of the extract have been reported by researchers, it was noted that the fresh leaves of *C.viscosa* are widely used as medicine for Jaundice.

Taxonomical profile

Kingdom: Plantea

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Capparales

Family: Capparaceae

Genus: Cleome

Specie: Viscosa

(UNEP-WCMC 2011)

Brief description of the plant

Cleome viscosa belonging to the family Capparaceae (Narayana*et, al.,* 2003). It is an annual sticky herb with strong penetrating odor. The plant is clothed with glandular and simple hairs (Rukmini, 1978). It is a widely distributed herbaceous plant with yellow flowers and long slender pods containing seeds, which is similar to that of mustard. *C.viscosa*, also called "Dog mustard", is a herb that

grow up to 1m height in India (Parimala*et, al.,* 2004). Cleome is a large genus included in the Capparaceae family, which comprises 427 species occurring in tropical and subtropical regions of the world (Brummit, 1992). The native of the plant is Africa and S. Arabia to Australia.The leaves are diaphoretic, rubefacient and vesicant. They are used as an external application to wounds and ulcers. The juice of the leaves has been used to relieve earache. The seeds are anthelmintic, carminative, rubefacient and vesicant. The seeds of *C.viscosa* are used to treat fever, diarrhea, and infantile convulsion (Rashmi*, et al.,* 2012). *C.viscosa* is a popular remedy for a variety of ailments as documented in Ethno-botanical surveys and traditional systems of medicine, such as Ayurveda, Siddha and Unani (Raghavan, 1993).

1.1 Importance of phytochemicals

The defuttered extract were tested for the presence of different secondary plant metabolites. Basic phytochemical screening consist of performing simple chemical test to detect the presence of alkanoids (Herbone, 1973),Tanins (Herbone, 1998) and saponid and cardial glycoside.

Observation of antibacterial activity may be due to the presence of potent phytochemical constituents in the extracts. The data obtained in the present work will be useful in the synthesis of new drugs of pharmaceutical importance. Phytochemical analysis or screening can be useful to substantiate and authenticate drugs e.gpharmacognostic

1.2 Statement of the problem

The chemical pollution of soil has become a major source of concern and has posed serious health problem within the last few years in many developed and developing nations (Ahmadpour*et, al.,* 2010). The effect of heavy metal on plant resulted inhibition, structural damage, a decline of physiological and biochemical activities, as well as of the function of the plant (Oancea, 2005)**.** As a weed, *C.viscosa*is found in abundance within the University premises including students hostels and staff quarters, road-site, University mini market and other areas. The plant is increasingly dominating agricultural lands and hence pose danger to other plant species.

1.3 Justification

This plant specie is considered as a weed. Phytochemicals are generally used as a essential nutrient responsible for the protection and carrying of many health situation, including fever, diarrhea, infantile convulsion, ulcer and earache e.t.c. Hence it is important to determine the specie chemical compositions present in the leaf, pod and roots.

1.4 Aim of the study

The aim of the study is to determine and identify some phytochemicals present in the leaf, root and pods of *C. viscosa.*

1.5 objectives of the study

This study have the following objectives;

1. To determine phytochemicals presence in the root, leaves and pods of *C. viscosa.*

2. To evaluate the difference concentrations of the secondary metabolites in the root, leaves and pods of *C. viscosa*.

CHAPTER TWO

2.0 Species and Origins

Spider plant (*Cleome or Gynandropsis* spp.) also commonly known as spider flower plant, African spider flower or cats' whiskers, comprises 150-200 species of which 50 are indigenous to Africa (Schippers, 2002). Edible and neglected species includes *C. allamani, C. hirta(Klotzsch) Oliv., C. gynandra* L. Chiov, *C. monophylla* L., *C. rutidosperma* DC, and *C. viscosa* L. The crop belongs to the Capparaceae family and according to Jansen 2004 and Mnzava and Ngwerume 2004, *C. hirta (Klotzsch) Oliv*. and *C. viscosa* originate from Ethiopia, Somalia, and through Eastern and Central Africa. *C. monophylla* is widespread in tropical and subtropical Africa. The origins of *C. allamani, C. gynandra*, and *C. rutidospermaa*re unknown.

Spider plant is a C4 plant capable of withstanding high daytime temperatures, intense sunlight, and drought.

2.1 Breeding

The plant is monoecious and has three types of flowers:

a) Flowers with anthers shedding pollen before stigma are receptive (protoandry). In this case, pollination can still occur on the same plant, but not on the same flowers.

b) Flowers with anthers shedding pollen when the stigma are receptive. This situation favors self- pollination.

c) Flowers with stigmas receptive before pollen is shed (protogyny). Depending on the type of flowers found on a particular genotype, selfing would then become a difficult task.

The crop has both purple and green stems. Difficulties reported in creating homogenous purple or green stem lines might be related to flower biology. In Venezuela, male sterile genotypes that could serve as female have been identified, but to date these have not been described in Africa.

Purple stem cultivars are reported to be more nutritious than those with green stems. They are also reported to be more resistant to insects, but more susceptible to diseases (Schippers, 2004).

Spider plant is consumed in most African and several South Asian countries. In Africa it is generally collected from the wild, although there is some limited cultivation of commercial leafy varieties in several countries in East and Southern Africa (Garino, 1997). Tender leaves, stems, pods, and flowers are consumed as vegetables by boiling in water or milk, alone or with other vegetables (e.g. tomato) and spices. To leach away the bitter taste, the initial cooking water is drained and fresh boiling water added. The small, aromatic leaves of *C. hirta* can be used as a spice

2.2 High in Vitamins and Micronutrient and Values

Spider plant is nutritious. It is known to contain high levels of beta-carotene, vitamin C, and moderate levels of calcium, magnesium, and iron. Regarding vitamin A content, an analysis carried out in Tanzania showed that the in vitro accessibility of all-trans-β-carotene in spider plant was the highest (26%) compared with cowpea, amaranth, sweet potato leaves, pumpkin, or combinations of these vegetables. The study also showed that when spider plant leaves were cooked with oil, in vitro accessibility

increased to 53%. However, the total amount of all 9- cis β-carotene did not significantly increase with the addition of oil (Mulokozi, andHedrenScanberg, 2004). The analysis showed that spider plant could contribute 72% (without addition of cooking oil) to 477% (with addition of cooking oil) of the daily vitamin A requirement. The daily requirement for children is set at 400 µg RE with an assumption that 50% of all accessible β-carotene will be converted to retinol in mucosa (Mulokozi, andHedrenScanberg, 2004). The weight of a vegetable portion consumed varied from 52 to 157 g, with a median weight of 84 g used as the basis for calculations.

The plant contains high crude protein, lipids, and phenolic compounds (Lyimo,et, al. 2003). The amino acid profile in spider plant is better than groundnut (Glewet, al. 2000), as all amino acid contents are higher.

Protein consumption can be compromised by consuming food containing elevated levels of trypsin, which inhibits proteases activities. A study by (Vanderjagt et, al. 2000) showed that trypsin inhibitor activity in spider plant was low (0.45 and 0.32 µg/mg) dry weight of plant respectively before and after boiling for 5 min

compared with the soybean reference (1.32 and 1.03 µg/mg) dry weight of plant respectively before and after boiling for 5 min. For comparison, the same study showed 12 leafy vegetables including amaranth (Amaranthusspinosus) consumed in Niger had inhibitory activities higher than that of soybean, and that these enzymes were resistant to heat.

Vegetables can lose their vitamin C content after cooking, but (Nodsi et, al. 1983) showed that spider plant best retains vitamin C compared with other vegetables. In fact, when 20 g of spider plant are cooked in 100 mL (very little) or 400 mL (excess) water, the losses were 5.3% and 18.3% respectively. By comparison, losses for amaranths A. graecizans were 86.2% and 46.4%, and A. spinosus 96.5% and 67.0%; for Ethiopian mustard (Brassica juncea), 51.4% and 86.1%; Moringa (Moringaoleifera) 85.4% and 98.5%; and bitter lettuce (Launaeacornuta) 93.5% and 94.5%. Understanding why spider plant retains most of its vitamin C after cooking would help indigenous vegetable breeders improve the nutritive value of this vegetable.

Free radicals are responsible for “oxidative stress” and often are implicated in the expression of several human diseases including diabetes, cancer, coronary heart diseases, neurodegenerative ailments, rheumatoid arthritis, etc. The human body has an antioxidant defense system that is believed to be strengthened by antioxidant-rich diets. Antioxidants include β-carotene (pro-vitamin A caretonoids) and vitamin C, which are present in fruits and vegetables.(Stangeland et, al. 2009) analyzed antioxidant activity in 35 Ugandan fruits and vegetables and found that spider plant had an antioxidant activity of 0.53 to 2.92 mmol/100 g and the derived food was a major contributor to the total dietary antioxidant capacity in the Ugandan diet.

2.3 High Oil Content in Seed

Seed of *C. viscosa* has high levels of polyunsaturated oils that can reach up to 29.6% (Mnzava, 1990). The oil can be extracted by simple pressing and does not require refining. The seed cake can be used for animal feed, and the seed itself for feeding birds (Mnzava andNgwerume, 2004).

2.4 Spider Plant as Medicine

According to ethno-pharmacological surveys, spider plant has a number of medicinal uses. In Uganda, the plant is used to induce labor during childbirth (Oryem – Origa, 2007). After giving birth, some women consume *C. visosa* to increase lactation and blood formation. Spider plant remedies are used to alleviate migraine, vomiting, diphtheria, vertigo, headache, pneumonia, septic ears, and stomach ailments; the plant also is used as an eyewash and fed to boys after circumcision (Kokwaro, 1993);Gessler(1994) analyzed 43 plants in Tanzania claimed to have medicinal properties and found that 37% of them had antimalarial activity and some had an IC50 as low as 10 µg/mL. For spider plant, the ethyl acetate extract was the most effective with an IC50 obtained with 14 µg/mL. In Rwanda, (Boily and Van Puyvelde, 1986) showed that methanolic extracts of spider plant could inhibit Candida albicans and Mycobacterium smegmatis at 50 mg/mL. Anotherstudy carried out in Uganda (Apio, et, al. 2003) showed that Staphylococcus aureus and Bacillus subtilis were susceptible to inhibition to methanolic extracts.

Experimental rats suffering from arthritis were administered ethanolic spider plant leaf extracts at a dose of 150 mg/kg of body weight for 30 days. Analysis of enzymes involved in the expression of arthritis showed that the rats had recovered from the disease and their status was comparableto the healthy control rats (Subramanian et, al. 2007). The control of the disease was related to substances present in the leaf extracts, including saponins, glycosides, lectins, steroids, flavonoids, tannins, triterpens, resins, phenolic compounds, and arthroquinones. However, the individual involvement of these compounds in the control of the disease needs to be investigated. In another experiment administering spider plant leaf extracts to rats expressing severe arthritis, the analysis of lipid peroxidases, catalases, glutathione peroxidase (enzymes involved in the scavenging of free radicals) showed that these enzymatic activities had increased significantly in the diseased rats compared with the control diseased rats that were not fed the leaf extract treatment (Kandaswamy et, al. 2005). On the other hand, the level of enzymes generating free radicals (glutathione and superoxide dismutase) was reduced significantly in the treated rats.

Free radicals also are cited as involved in the expression of plant diseases. Examining whether spider plant is effectively less susceptible to diseases than other plants (by using spider plant mutants that do not synthesize enzymes involved in the scavenging of free radicals) would be a useful model for plant pathologists.

2.5 Uses in Traditional Medicine and other reported Activities

In Ayurvedic system of medicine, the plant is used in fever, inflammations, liver diseases, bronchitis and diarrhea (Chatterjee and pakrashi1991). The rural people use the fresh juice of the crushed seed for infantile convulsions and mental disorders. The juice of the plant diluted with water is given internally in small quantities in fever and the leaves are useful in healing the wounds and ulcer (Nadkami, 1982). *C. viscosa* is highly effective in a wide spectrum of diseases and reported to possess antidiarrhoeal, analgesic, psycho-pharmacological, antimicrobial properties including in vitro Helicobacter pylori and wound healing activity (Parvathi *et, al.* 2011). However, available literature revealed that no detailed pharmacognostic studies have carried out on plant; hence the present investigation was undertaken. The object of present

study is to evaluate various pharmacognostical parameters such as macroscopy, microscopy physicochemical parameters, fluorescences analysis and phytochemical studies of the plant.

2.6 Spider Plant in Crop Protection

Spider plant has insecticidal and insect repellent properties. Spraying an aqueous extract of spider plant can considerably reduce aphid and thrip populations (Schippers, 2002). Intercropping spider plant with cabbage also reduces diamondback moth as well as thrip attacks (Schippers,2002). Intercropping spider plant in rose- producing greenhouses at 8.3 plants/m2 was reported to reduce red spider mite populations in Kenya (Nyalala and Grout 2007). The plant also was shown to have anti-tick properties (Hassan, 1992). Unpublished results obtained by AVRDC show that intercropping spider plant with tomato reduces thrips populations. Spider plant contains glucosinolates, including methylglucosinolate (Hasapis et, al. 1981), cleomin, andglucocapparin (Kjaer and thomsen 1963); their hydrolysis gives rise to methyl isothiocyanates, a strong antimicrobial compound (Lori et, al. 1993) that may contribute to insecticidal properties,

along with phenolic compounds and an acid- volatile oil present in the glandular (which are involved in the characteristic mustard smell). Glucosinolates are responsible for the bitterness of the leaves. To date, no research has been published on the use of spider plant to control plant diseases; this may be a promising direction for future indigenous vegetable production research.

CHAPTER THREE

3.0 Method and Materials

3.1 Study Area

This analysis was conducted in the Biochemistry laboratory, Usmanu Danfodiyo university Sokoto-Nigeria (Longitudes 11^0 30 to 13^0 50 E and Latitude 4^0 to 6^0 N).

3.2 Collection of Plant Samples

The plant material were washed with running tap water and then rinse with distilled water. The materials were shade dried and then grinded into powder using mortar and pestle sieved. The clopped leaves, roots and pods were collected from Clapperton road, Gawonnama area Sokoto.

3.3 Preparation of aqueous Extracts

25g of powder sample of leaves, roots and pods of *C. viscosa*would be added into a 250ml conical flask containing 125ml of distilled water, stirred and allowed to stand for 24hrs with occasional shaken. The mixture would be filter using whatman No1. Filter paper and then pass through a sterilize filter paper to avoid

any contamination. The procedure was repeated using, methanol, ethanol and chloroform. The filtered extracts was kept in sterilize screw capped bottle in a refrigerator until prior to the analysis.

3.4 Phytochemical Screenings

Phytochemical analysis was carried out to determine , tannins, saponins, glycosides, anthraquinone, steroids, balsams, flavonoids, alkaloids, volatile oils, phenolics, terpenes, amino acid, saponin glycosides and cardiac glycosides.

3.4.1 Test for Alkaloids

About 2ml of each extract is stirred with 2ml of 10% aquences hydrochloric acid. 1ml is treated with few drops of wagners reagent and second 1ml portion is treated similarly with mayers reagent. Turbidity or precipitation with either of these reagents is taken as preliminary evidence for the presence of alkaloids (Harbone, 1973).

3.4.2 Test for Saponins

5ml of the extracts will be placed in a test tube + 5ml of water and shaked strongly. The whole tube will be filled fronth that lasts for several minutes (Harbone, 1998).

3.4.3 Test for Flavonoids

3ml of aliquot filtrate and 1ml of 10% NaOH sodium hydroxide, if a yellow colour is developed this indicates the possible presence of flavonoid compounds (Harbone, 1998).

3.4.4 Test for Tannins

5% Ferric chloride solution will be added drop by drop into 3ml of the extract and colour produced is noted. Condensed tannins usually give a dark green colour, hydrolysable tannins give blue-black colour (Harbone, 1998)

3.4.5 Test for Amino Acid (Ninhydrin Test)

To the extract, 0.25% w/v ninhydrin reagent would be added and boiled for few minutes. Formation of blue colour indicates presence of amino acid (kokate, 1994).

3.4.6 Test for Phenols (Ferric Chloride Test)

5% Ferric chloride solution will be added drop by drop into 3ml of the extract and colour produced is noted. Condensed tannins usually give a dark green colour, hydrolysable tannins give blue-black colour (Harbone, 1998)

3.4.7 Test for Glycosides (Salkowski's Test)

2.5ml of 50% H_2SO_4 is added to $5cm^3$ of the extracts in a test tube. The mixture is heated in boiling water for 15minutes. Cool and neutralize with 10% NaOH, 5ml of fehlings solution is added and the mixture is boiled. A brick-red precipitate is observed which indicate the presence of glycosides (Harbone, 1973).

3.4.8 Test for Terpenoids

5ml of extract and 2ml chloroform were mixed in a test tube. Then 3ml of concentrated sulphuric acid was carefully added slowly by the side of the test tube. Appearance of reddish brown coloration between upper and lower layer (interface) indicate presence of terpenoids (sofowara, 1993)

3.4.9 Test for Volatile Oils

1ml of the fraction was mixed with dilute HCL. A white precipitation was formed which indicate the presence of volatile oils

3.4.10 Test for Balsams

The extract was mixed with equal volume of 90% ethanol. 2 drops of alcoholic ferric chloride solution was added to the mixture. A dark green colour indicates the presence of balsams.

3.4.11 Test for Anthraquinone

0.5g of each plant extract is shaken with 10ml of benzene, 5ml of 10% ammonia solution is added. The mixture is shaken and the presence of a pink, red or violet colour in the ammoniacal (lower) phase indicates the presence of anthraquinones.

3.4.12 Test for Steroids

This will be carried out according to the method of Harbone 1973.

0.5g of the extract is dissolved in 2ml of chloroform 2ml of sulphuric acid is carefully added to form lower layer. A reddish

brown colour at the interface indicates the presence of a steroidal ring.

3.4.13 Test for Saponin Glycosides

2.5ml of the extract was added 2.5ml of fehlings solution A and B. A bluish green precipitate showed the presence of saponin glycosides.

3.4.14 Test for Cardiac Glycosides

To one of herb extract 2ml of 3.5% ferric chloride solution is added and allowed to stand for one minute. H_2S0_4 is carefully poured down the wall of tune so as form a lower layer. A reddish brown ring the interface indicates the presence of cardiac glycosides.

CHAPTER FOUR

4.0 Result

The plant aqueous extracts of the *Cleome viscose* was analysed qualitatively for the presence of phytochemicals such as alkaloids, tannins, saponins, flavonoids, glycosides, steroids, balsams, cardiac glycosides, saponin glycosides, anthraquines, volatile oils, terpenoids, phenols and amino acids. The results are presented in table1.

It was found that most of the phytochemicals analysed were present in both leaves, roots and pods these includes amino acid, terpenoids, volatile oils, steroids, alkaloids and glycosidessaponin,cardiac glycosides and anthraquines were not observed in the pods extract, similarly Tannins, Flavonoid, cardiac glycosides saponin Balsam, Anthraquines and Phenols were absent in the root extracts.

Table 1: Phytochemical screening of leaves, pods and roots aqueous extract of *Cleome viscose L*

S/N		Leaves	Pods	root
1.	Tannins	+	+	-
2	Saponin	+	-	+
3	Flavonoid	+	+	-
4	Glycosides	+	+	+
5	Alkaloids	+	+	+
6	Cardiac glycoside	+	-	-
7	Steroids	+	+	+
8	Saponin glycosides	+	+	-
9	Balsams	+	+	-
10	Anthraquines	-	-	-
11	Volatile oils	+	+	+
12	Terpenoids	+	+	+
13	Phenols	+	+	-
14	Amino acid	+	+	+

Key: + Positive

- Negative

CHAPTER FIVE

5.0 Discussion

Plants are rich and useful source of primary and secondary metabolites like proteins, lipids and carbohydrates, alkaloids, flavonoids, terpenoids, tannins e.t.c. These metabolites are useful for the plant as well as for the human being for the treatment of various illnesses such as fever, diarrhea, earache, headache and external wounds etc (Nisha *et, al.,* 2011). The pods of *C. viscosa* are documented to be beneficial in helminthic infections such as convultions, fever and diarrhea. The roots are considered cardiac stimulant and are given internally in cases of snake bite and diabetes.

Determinations of these metabolites are helpful to know the medicinal as well as nutritional value of respective plants (Mandal *et, al.,* 2003). The present work have determine the content level of primary and secondary metabolite in the roots, pods and leaves of *C. viscosa* by using various chemical test as well as by applying the well developed analytical methods.

5.1 Conclusion

It can be concluded that the leaf aqueous extract of *C. viscosa* showed high content of phytochemicals, all of the secondary metabolites were found to be present with the exception of Anthraquinines. The pods aqueous extract showed presence of all the phytochemicals tested with the exception of Saponin, Cardiac glycoside and Anthraquinines. Roots extract was found to have the least number of phytochemicals studied.

5.2 Recommendation

Further studies need to be done using different extract (ethanol, methanol etc) to be able to isolate, identify and characterize the phytochemicals.

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