ANALYSIS OF PHYTOCHEMICAL CONSTITUENT OF MANGO (*Mangifera iudica*) LEAVES AND STEM BARK

BY

MUHEEZAT MUHAMMED MAM
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DECLARATION

I hereby declare that this project is written by me and has not been presented before in any application for Bachelor Degree except for quotations and summaries which have been dully acknowledged

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Muheezat Muhammed Mam

Date

DEDICATION

This work is dedicated to Almighty Allah for his guidance and to my parents in person of Engr. Muftau Aderibigbe and Fausat Aderibigbe for their prayers, encouragements, and support.

CERTIFICATION

This project entitled "Analysis of phytochemical constituent of mango (*mangifera indica)* leaves and stem bark" meets the regulation governing the award of Bachelor of Science of the Federal University Gusau and is approved for its contribution to knowledge and literary presentation.

.....	.....
Mal. N. Suleiman	Date
(Supervisor)	
.....	.....
Prof. S. J. Oniye	Date
(Head of Department)	
.....	.....
Prof. K. Abdullahi	Date
(Dean of Science)	
.....	2/12/2019
Prof. W. S. Japhet	Date
(External Examiner)	

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ABSTRACT

Mango (*Mangifera indica)* is one of the most important fruits plant in the Anacardiaceae family and is a delicious fruit which is high in different phytochemicals. The research was carried out to identify the phytochemical constituents of mango (*Mangifera indica)* leaves and stem bark using different solvent for extraction. The result obtained showed the presence of flavonoids, tannins, cardiac glycosides, steroids, and balsams. This research confirms the presence of phytochemicals in both the leaves and stem bark of mango.

CHAPTER ONE

1.0 Introduction

The Mango (*Mangifera indica*), is one the most economically important fruit plant in the Anacardiaceae family. Other important members of this family include cashew, pistachio, and the mombins (Spondias spp.). The family have 73 genera and about 600-850 species, with a few representatives in temperate regions, distinguished by their resinous bark and caustic oils in leaves, bark, and fruits. Apart from edible fruit Anacardiaceous species also yield other valuable products like wood, gums and resins, wax and varnishes and tanning materials. (Whitmore, 2003). The mango plays an important part in the diet and cuisine (a characteristic style of preparing food) of many diverse cultures. There are over 1000 named mango varieties (*Mangifera foetida, Mangifera caesia, Mangifera casturi, Mangifera sylvatica, Mangifera laurina, Mangifera odorata, Mangifera zeylanica, Mangifera pajang, Mangifera pentandra, Mangifera indica*) throughout the world, which is a testament to their value to humankind. Mango is a common garden tree throughout the tropics. When ripe, this delicious dessert fruit is particularly high in vitamin A. The fruit is also eaten green, processed into pickles, pulps, jams, and chutneys, and is frozen or dried. The mango fruit is also an important source of sustenance for birds, bats, insects, and mammals (Nahesh*et al.*, 2013).

The history of Mango began thousands of years ago on the Indian sub-continent. The Mango is the national fruit of India, Pakistan and the Philippines. It is also the national tree of Bangladesh. Not only is it one of the most highly prized fruits of South Asia, it is also intimately connected with folklore and legends across many religions (Mukhejee, 2001). Mango extracts from leaves, fruit, seed kernel, fruit pulp, roots, and stem bark have been used extensively for medicinal purposes in many

countries. The ethno medicinal use of mango stem bark aqueous extract in Cuba has been documented widely. It has been extensively used in cancer, diabetes, asthma, infertility, lupus, prostatisis, prostatic hyperplasia, gastric disorders, arthralgies, mouth sores and tooth pain (Tola, 2009).Mangiferin is the major component in mango stem bark. Other flavonoids and flavonol constituents include quercetin, catachin and epicatechin. The phytochemical screening of the raw mango showed that polyphenols, terpenoids, sugars and saponins were present in mango stem bark. Many phenolic constituents, benzoic acids and its propylester, three free sugars (galactose, glucose and arabinose) and three polyalcohols (sorbitol, myoinositol and xylitol) were identified and tested from the mango fruits and stem bark related constituents of mango stem barks. Volatile components of mango stem bark were extracted, and the major constituents identified were β-relemens, aromandrene, α-guaiene, β-endesmol, β-sitrosterol and β-campesterp. The amount of sesquiterpenoid hydrocarbons was higher than the oxygenated compounds. The composition of the fatty acid fraction was also determined as their trimethylsilyl esters derivatives and the major constituents were palmitic, oleic and linoleic acids with a minor proportion of myristic and estearic acids. Many polyunsaturated and dicarboxylic acids of biological importance such as eicosatriencoic, succinic and malonic were found to be present in mango stem bark in trace levels (Abdullah.,2010).

*Mangifera indica*stem bark has antioxidant, anti-inflammatory and immunoduletory properties and have been developed as nutritional supplement or functional food in several formulations (antioxidant) or anti-inflamatory, analgesic (tablet, capsule, syrup and cream) and have been extensively used to prevent disease progress or improve the patient's quality of life in diseases like cancer, asthma, gastric and dermatological disorders.

1.1 Statement of the problem

Consumption of mango fruits has immensely increased among world populous. Many disease outbreaks such as cholera and diarrhea are strongly attributed to consumption of mango as mostly unhygienic. Too much consumption can prevent us from being in caloric deficit, thus can impend our weight loss journey. Gastrointestinal problems: excessive consumption of mangoes especially unripe mangoes can be responsible for gastrointestinal problems like indigestion.

1.2 Aim and objective

The aim of this work is to identify the phytochemical constituents of mango extract and the objective is;

To identify the various phytochemicals, present in mango leaves and stem bark.

1.3 Justification

Mango as one of the common fruits in most part of the world has a lot of bioactive compounds unknown to many people. Exploring this bioactive element will provide a valuable information to industries, pharmaceutical companies and young researchers as well.

1.4 Research Question

What are the phytochemical constituents present in the leaves and stem bark of *Mangifera indica*?

1.5 Hypothesis

There is no phytochemical constituent in *Mangifera indica* leaves and stem bark.

CHAPTER TWO

2.0. Literature review

The plantkingdom represents a treasure trove of structurally diverse bioactivemolecules. Most of the best plant medicines are the sum of their constituents.Thetherapeutic, beneficial and physiológical effects of plant materialstypicallyresult from the mixing of these secondary products present inthe plant. As more phytoconstituents are being identifiedand tested, traditional uses of the plants are being verified (Wakeel *et al.*,2013).

The stem bark and leaves have astringent prosperities and are used in Nigeria as lotion to relieve toothache, sore gums, sore throat or as an infusion in malaria, diarrhea and dysentery treatment. All the organs of the plants are rich in tannins and flavonoids (Raaman, 2006). The mango tree is rich in phytochemicals, which are vital in health promotion, disease prevention and drug production. Phytochemicals act as antioxidant, stimulate the human system, induce protective enzymes in the liver or block damage to genetic materials. Phytochemicals exhibit a wide range of biological functions due to their antioxidant properties. Several types of polyphenols (phenolic acid hydroly-sable tannins and flavonoids) found in mango show anti-carcinogenic and anti-mutagenic effects. Polyphenols interfere in many steps of malignant tumors, inactivating carcinogens, inhibiting the expression of mutagens and the activity of enzymes involved in the activation of procarcinogens.

2.1.0 Phytochemicals

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Some of the well-known phytochemicals are lycopene in tomatoes, isoflavones in soybean, flavonoid in fruits etc. According to World health organization (WHO), a medicinal plant is any plant which in one or more of its organ

contains substance that can be used for the synthesis of useful drugs (WHO. 1977). Biologically, active chemical substance such as saponins, tannins, essential oils flavonoids, alkaloids, and other chemical compounds are found in the plant organ(Sofowora, 2012)

2.1.1. Description of Flavonoids

The flavonoids and phenolic compounds are of interest because they are important to human as a class of antioxidants and health promoting compounds present in vegetable food. Flavonoids are potent water soluble antioxidants and free radical scavengers that prevents oxidation of cell damage, have strong anti-cancer activity and protects against all stages of carcinogenesis(Sofowora, 2012). Hundreds of phytochemical compounds, with several different biological functions, have been identified in plant-based foods. Therefore, consuming a variety of plant-based foods helps to ensure that individuals receive the optimum benefits from the fruits and vegetables consumed (Manach*et al.*, 2004).

2.1.2 Description of Balsams

Balsams are not as fluid as oleoresins but are relatively soft and initially malleable which enables them to be used as an ointment in wound healing. Balsams consist primarily of cinnamic and benzoic acids and are therefore considered to be a phenolic resin (Tiwari *et al.*, 2011). The volatile fraction tends to be fragranced and is therefore used in perfumery and cosmetics, and burned as incense. It is mostly found in the stem barks of most plant and can be also found in the leaves of plant like foxglove (Raaman, 2006).

2.1.3. Description of Tannins

Tannins are soluble in water and alcohol and are found in the root, bark, stem and outer layers of plant tissue. Tannins are used as antiseptic and this activity is due to

presence of the phenolic group. Common examples of hydrolysable tannins include the aflavins(From tea), daidezein, geinstein and glycitein. Tannin-rich medicinal plants are used as healing agents in a number of disease. In Ayurveda. formulations based on tannin-rich plants have been used for the treatment of diseases like leucorrhoea, rhinnorhoea and diarrhea (Kar, 2007).

2.1.4. Description of Saponins

Saponins have a bitter taste. Some saponins are toxic and are known as sapotoxin. Saponins have many health benefits, studies have shown that saponins have antitumor and anti-mutagenic activities and can lower the risk of human cancers, by preventing cancer cells from growing. Saponins seem to react with the cholesterol rich membranes of cancer cells, thereby limiting their growth and viability (Tiwari *et al.*, 2011).

2.1.5. Description of Cardiac glycosides

Cardiac glycosides are a group comprising two main classes of compounds that differ in the structure of their aglycone. Cardiac glycosides are with a basic nucleus of cyclopentanoperhydrophenanthrene substituted. Plants can produce both cardenolides and bufadienolidess in both the flowers, root, seeds and leaves of some herbs (Raaman N, 2006). Digoxin and digitoxin are the two most widely used digitalis inotropes. There are two million patients receiving these cardenolides in the US. In general, some isocardenolides appeared to be devoid from any cardiac activity (Tiwari *et al.*, 2011).

2.1.6 Description of Alkaloids

Alkaloids are one of the largest groups of plant secondary metabolites, being present in several plant families. Alkaloids encompass neuroactive molecules, such as caffeine and nicotine, as well as life-saving medicines including emetine used to fight

oral intoxication and the antitumorals vincristine and vinblastine. Alkaloids can act as defense compounds in plants, being efficient against pathogens and predators due to their toxicity (Tiwari *et al.*, 2011).

2.1.7. Description of Volatile oil

Volatile oils are mixture of hydrocarbon terpenes, sesquiterpenes and polyterpenes and their oxygenated derivatives obtained from various parts of the plant. Volatile oils evaporate on exposure to air at ordinary temperature and are the odorous constituents. As volatile oil oils are responsible for the essence or odor of the plant they are also known as essential oils (Tiwari *et al.*, 2011).

2.1.8. Description ofAnthraquines

Anthraquinones are the main active constituents in herbs often used to relieve constipation. They have an irritant or stimulating laxative effect on the large intestine. They constitute the largest group of naphthoquinones, and are often red or purple in pigment (Tiwari *et al.*, 2011).

2.2.0. Extraction of phytochemicals

2.2.1. Maceration Method

The whole powdered material is allowed to contact with the solvent which is in a stoppered container for a particular time period with frequent agitation. At the end of the process the solvent is drained off and the remaining micellar is removed from the plant material through pressing or centrifuging. Maceration is not an advanced technique since active ingredients cannot be totally extracted (Majeokodumi, 2015).

2.2.2. PercolationMethod

A percolator which has a narrow cone shaped vessel open at both ends is used for this technique. The plant material is moistened with the solvent and allowed to place in a percolation chamber. Then the plant material is rinsed with the solvent for several

times until the active ingredient is extracted. The solvent can be used until its point of saturation (Majeokodumi, 2015).

2.2.3. Soxhlet ExtractionMethod

This method is widely used when the desired compound has a limited solubility in the particular solvent and impurities are less soluble in the solvent. The finely ground sample is placed ina porous bag or "thimble" which made out of filter paper or cellulose. The solvent which the desired compounds are going to extracted is kept in the round bottom flask (Majeokodumi, 2015).

CHAPTER THREE

3.0 Materials and methods

3.1.0. Study area

The research was conducted at Federal University Gusau in Biochemistry Laboratory.

3.2.0. Sample collection

Fresh leaves and stem bark of mango were collected from Federal University Gusau behind Plant Science and Biotechnology laboratory using hands to pick the leaves and a sharp knife was used to scrap the stem bark. The leaves and stem bark were collected in a separate clean polythene bag and taken to Biochemistry Laboratory.

3.3.0. Sample preparation

Fresh collected leaves and stem bark of *Mangifera indica* were air dried for 24 hours at normal room temperature. After air drying, both samples were introduced into the oven for drying at 105°C, the dried samples were grounded using mortar and pestle into powder. 5g of the sample was soaked in 100ml of distilled water for 24 hours and sieved.

3.4.0. Extraction of phytochemicals

The crude powder of the leaves and stem bark were subjected to qualitative phytochemical analysis.

3.4.1. Determination of alkaloids in mango Leaves and Stem Bark

Two mill of each extract was stirred with 2ml of 10% aqueous HCL. One ml was treated with a few drops of wagner`s reagent and the second, 1ml portion was treated similarly with mayer`s reagent. Turbidity or precipitation with either of these reagents was taken as a preliminary evidence for the presence of alkaloids (Okwu *et al.*, 2007).

3.4.2. Determination of flavonoids in mango Leaves and Stem Bark

Three mill of the extract was added in 1ml of 10% NaOH. A yellow color indicates the possible presence of flavonoid compounds (Okwu *et al.*, 2007).

3.4.3 Determination of tannins in mango leaves and stem bark

Five percent ferric chloride solution was added drop by drop in 2-3ml of the extract. Condensed tannins usually give a dark green color while hydrolysable tannins give blue-black color (Okwu *et al.*, 2007).

3.4.4. Determination of saponin glycosides in mango Leaves and Stem Bark

To 2.5 ml of the extract, 2.5ml of fehling`s solution A and B was added. A bluish green precipitate shows the presence of saponin glycosides (Okwu *et al.*, 2007).

3.4.5. Determination of steroids in mango Leaves and Stem Bark

Three mill of the extract was dissolved in 2ml of chloroform. 2ml of sulphuric acid was careful added to form a lower layer. A reddish-brown color at the interface indicates the presence of a steroidal ring (Okwu *et al.*, 2007).

3.4.6. Determination of saponins in mango Leaves and Stem Bark

Five mill of the extract was placed in a test-tube, 5ml of water was added and shake properly. The presence of froth in the whole test-tube that last for several minutes indicates the presence of saponin (Okwu *et al.*, 2007).

3.4.7. Determination of glycosides in mango Leaves and Stem Bark

Fifty percent H_2SO_4 was added to 5ml of the extract in a test-tube. The mixture was heated in a boiling water for 15 minutes. Cool and neutralize with 10% NaOH. 5ml of fehling`s solution was added and the mixture was boiled again. Presence of a brick-red precipitate indicates the presence of glycosides (Okwu *et al.*, 2007).

3.4.8. Determination of cardiac glycosides in mango Leaves and Stem Bark (keller killian`s test)

To one of the extract, 2ml of 3.5% ferric chloride solution is added and allowed to stand for one minutes. H_2SO_4 is carefully poured down the test tube so as to form a lower layer. A reddish brown ring, the interface indicates the presence of cardiac glycoside (Okwu *et al.*, 2007).

3.4.9. Determination of balsams in mango Leaves and Stem Bark

Three mill of the extract was mixed with 3ml of 90% ethanol. Two drops of alcoholic ferric solution were added to the mixture. A green color indicates the presence of balsams (Okwu *et al.*, 2007).

3.4.10. Determination of anthraquinesin mango Leaves and Stem Bark

Three mill of the extract was shaken with 3ml of benzene, and 3ml of 10% ammonia solution was added, and the mixture was shaken again. The presence of a pink, red or violet color in the lower phase indicates the presence of anthraquines (Okwu *et al.*, 2007)

3.4.11. Determination of volatile oil mango Leaves and Stem Bark

One mill of the extract was mixed with diluted HCL. A white precipitate was formed which indicates the presence of volatile oils (Okwu *et al.*, 2007).

CHAPTER FOUR

4.1.0 Result

The phytochemical analysis of mango leaves and stem bark shows the presence of flavonoids in the leaves using distilled water and absent using methanol and chloroform. It shows the presence of flavonoids in stem bark using distilled water and chloroform and absent using methanol. It shows the presence of tannins and cardiac glycosides in the leaves using distilled water and absent using methanol and chloroform, it shows the of tannin in the stem bark using both distilled water and methanol and absent using chloroform while cardiac glycosides are present using methanol and absent using distilled water and chloroform. Saponin, glycosides, saponin glycosides, alkaloids, anthraquines and volatile oil are absent in the leaves and stem bark using distilled water, methanol and chloroform. Steroid is present in the leaves using methanol and absent using distilled water and chloroform. Steroid is absent in the stem bark using distilled water, methanol and chloroform.Balsams is present in the stem bark using distilled water, methanol and chloroform. Balsam is present in the leaves using chloroform and absent using distilled water and methanol.

le 1: Phytochemical content of mango leaves and stem bark using distilled water

Phytochemicals	Leaves	Stem Bark
Flavonoids	++	++
Tannins	++	++
Saponins	–	–
Glycosides	–	–
Cardiac glycosides	++	–
Saponin glycosides	–	–
Alkaloids	–	–
Steroids	–	++
Balsams	–	–
Anthraquines	–	–
Volatile oil	–	

Keys: ++ = present

– = Absent

e 2: Phytochemical content of mango leaves and stem bark using methanol

Phytochemicals	Leaves	Stem Bark
Flavonoids	++	–
Tannins	++	++
Saponins	–	–
Glycosides	–	–
Cardiac glycosides	–	–
Saponin glycosides	–	–
Alkaloids	–	–
Steroids	++	–
Balsams	–	++
Anthraquines	–	–
Volatile oil	–	–

Keys: ++ = present

– = Absent

Table 3: Phytochemical content of mango leaves and stem bark using chloroform

Phytochemicals	Leaves	Stem Bark
Flavonoids	–	++
Tannins	–	–
Saponins	–	–
Glycosides	–	–
Cardiac glycosides	–	–
Saponin glycosides	–	–
Alkaloids	–	–
Steroids	–	–
Balsams	++	++
Anthraquines	–	–
Volatile oil	–	–

Keys: ++ = present

– = Absent

Table 4: Comparative Phytochemical Content Of Mango Leaves And Stem Bark

Phytochemicals	Distilled Water		Methanol		Chloroform	
	Leaves	Stem Bark	Leaves	Stem Bark	Leaves	Stem Bark
Flavonoids	++	++	–	–	–	++
Tannins	++	++	–	++	–	–
Saponins	–	–	–	–	–	–
Glycosides	–	–	–	–	–	–
Cardiac glycosides	++	–	–	++	–	–
Saponin glycosides	–	–	–	–	–	–
Alkaloids	–	–	–	–	–	–
Steroids	–	–	++	–	–	–
Balsams	–	++	–	++	++	++
Anthraquines	–	–	–	–	–	–
Volatile oil	–	–	–	–	–	–

Keys: ++ = present

– = Absent

CHAPTER FIVE

5.0 DISCUSSION

This research work confirmed that mango leaves and stem bark contained many phytochemicals using different extraction method.

The research is not in accordance with the finding of Raaman N, (2006), who reported that all organs of the plants are rich in tannins and flavonoids, because tannins and flavonoids is not found in the leaves and stem bark using methanol and also absent using chloroform but present in stem bark using chloroform extract.

The research is in accordance with the findings of Sofowora S.D, (2012), biologically, active chemical substances such as Saponin, tannins, essential oils, flavonoids, alkaloids and other chemical compounds are found in the plant organs.

The finding is in line with the findings of Kar, (2007) who stated that tannins are soluble in water and alcohol and are found in root, bark, stem and outer layer of plant tissues. Because tannins are present in distilled water extract than all the extract used.

The research confirmed the absent of saponin, glycoside, saponin glycoside, alkaloids, anthraquines and volatile oil using all the extract used and in accordance with the work of Raaman N, (2006), Tiwari et al. (2011), that saponin, glycoside, saponin glycoside, alkaloids are all found in some herbs. This may be the reason for their absence in mango plant.

5.1.0 CONCLUSION

From this research, it is found that distilled water is more effective in phytochemical extraction because six phytochemicals were identified using distilled water followed by methanol extract with four phytochemicals and three phytochemicals using chloroform.

5.2.0 RECOMMENDATION

Further research should be conducted on phytochemical content of mango flower, root and seed.

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