QUANTIFICATION OF PHYTOCHEMICAL CONTENTS AND ANTIMICROBIAL ACTIVITY OF *Plectocomiopsis geminiflora*

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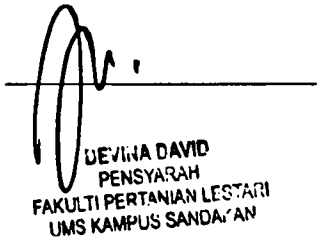

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ABSTRACT

This study was carried out at the Faculty of Sustainable Agriculture, Universiti Malaysia Sabah (UMS) Sandakan Campus from July 2015 to September 2015 to study the effect of different extraction solvents on the extraction yield, qualitative and quantitative phytochemical contents and antimicrobial activity of *Plectocomiopsis geminiflora*. Aqueous ethanol used for extraction solvent showed the highest extraction yield as compared to aqueous methanol and distilled water. For the qualitative analysis, distilled water extract indicates more presence of phytochemicals as compare to aqueous ethanol and aqueous methanol. From this analysis, the result indicates the presence of saponins, glycosides, flavonoids, steroid, phenols, alkaloids, tannins and terpenoids and the absence of quinones and anthocyanin. For the quantitative analysis, distilled water extract showed the highest total of phenolic and alkaloid content while for flavonoid content, methanol extract showed highest amount. The antimicrobial activity which was focused only into the antibacterial activity has been done using disc diffusion technique. From this experiment, it revealed that only *Bacillus cereus* was susceptible to all three extraction solvents while others bacteria such as *Eschoria coli, Salmonella thypii*, and *Staphylococcus aureus* only susceptible to distilled water extract. This indicates the potential usefulness of this plant in the treatment of various pathogenic diseases in this locality.

KUANTIFIKASI KANDUNGAN FITOKIMIA DAN AKTIVITI ANTIMIKROB *Plectocomiopsis geminiflora*

ABSTRAK

Kajian ini dijalankan di Fakulti Pertanian Lestari, Universiti Malaysia Sabah Kampus Sandakan (UMSKS) dari Julai 2015 hingga September 2015 untuk mengkaji kesan pengekstrakan pelarut yang berbeza terhadap jumlah pengekstrakan, kualitatif dan kuantitatif kandungan fitokimia dan aktiviti antimikrob Plectocomiopis geminiflora. Akueus etanol yang digunakan sebagai pengekstrakan pelarut menunjukkan jumlah pengekstrakan yang tertinggi berbanding akueus metanol dan air suling. Untuk analisis kualitatif, ekstark air suling menunjukkan lebih banyak kehadiran fitokimia berbanding dengan akueus etanol dan akueus metanol. Dari analisis ini, fitokimia yang hadir adalah saponin, glikosida, flavonoid, steroid, fenol, alkaloid, tannin dan terpenoid dan ketakhadiran kuinon dan antosianin. Untuk kuantitatif analisis, ekstrak air suling menunjukkan jumlah kandungan fenolik dan alkaloid yang tertinggi manakala untuk jumlah kandungan flavonoid, ekstrak metanol menunjukkan jumlah yang tertinggi. Aktiviti antimikrob yang mana fokus kepada aktiviti antibakteri telah dijalankan menggunakan teknik difusi disk. Dari ekperimen ini, ia menunjukkan bahawa hanya Bacillus cereus sahaja yang rentan terhdap ketiga-tiga pengekstrakan pelarut, manakala bakteria yang lain seperti Eschoria coli, Salmonella thypii, dan *Staphylococcus aureus hanya rentan terhadap ekstrak air suling sahaja. Ini menunjukkan, tumbuhan ini mempunyai kadar potensi yang berguna dalam pelbagai rawatan penyakit patogen dalam kalangan penduduk tempatan.*

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

g	Gram
mg	Milligram
cm	Centimeter
mm	Millimeter
L	Litre
ml	Milliliter
µl	Microliter
°C	Degree Celcius
%	Percentage
H_2SO_4	Sulphuric acid
NaOH	Sodium hydroxide
HCl	Hydrochloric acid
$FeCl_3$	Ferum
Na_2CO_3	Sodium carbonate
nm	Nanometer
M	Molar
Na_2HPO_4	Sodium phosphate
BCG	Bromocresol Green
ANOVA	Analysis of Variance
SPSS	Statistical Package for Social Science
GAE	Gallic Acid Equivalent
QE	Quercetin Equivalent
AE	Atropine Equivalent

CHAPTER 1

INTRODUCTION

1.1 Background of study

Vegetables should be an important part of humans' daily diet. In everyday usage, vegetables are consumed by humans as food, as part of an appetizing course or meal. Generally, people consume vegetables is because to maintain or increase their body health. Besides the commonly consume vegetables, some underutilized vegetables are also important to be add in the diet especially for those in rural communities that hardly to get food sources and mostly depend on natural resources. These natural resources of underutilized vegetables have become the solution for food security problem that might be encountered by the rural communities especially in Borneo. Borneo is the biggest part in Malaysia which also has a rich diversity of underutilized vegetables that grow wildly in the tropical rainforest of each region in Borneo (Sabah and Sarawak).

At the present time, several underutilized vegetables have been cultivated and selling out not only to the local communities but also has been consumed by other communities of an urban area. In this study will only focus on one type of underutilized vegetables of Sabah which is typically known as *Ambarua* by Murut peoples in Sabah and the scientific name of this vegetable is *Plectocomiopsis geminiflora* (Kulip, 2003). However, this underutilized vegetable also can be found in Sarawak which is known as *wi lalis* in the Iban culture and *mudmua* by the Dayak communities. Ambarua is light yellow in color and has a bitter and slightly sweet taste; its tree can grow up to 30 m high (Zabidah *et al.*, 2014). This vegetable can be consumed by cooking the stem's shoot.

Usually the consumption of vegetables are because of the wide range of the biologically active, non-nutritive compounds known as phytochemicals. Phytochemicals have been described as bioactive , non-nutrient compound in fruits, vegetables, grains and other plant food that have been linked to reducing the risk of major degenerative diseases (Liu, 2004). They are also known as plant-derived chemicals, which are presence has been considered of crucial nutritional importance in the prevention of chronic diseases such as cancer, cardiovascular disease and diabetes (Aruoma, 2003).

In Malaysia and most of the emerging countries, a wide variation of underutilized vegetables are used by native people as part of their daily diets or as traditional medicines. For example, Tutan (Solanum nigrum L.) which is used to kill intestinal worms and reduce high blood pressure (Ng *et al.*, 2012). In addition, nutritional composition study on the indigenous fruits and vegetables in Sarawak also showed that most local fruits are high in protein and potassium, while the nutritional value of indigenous vegetables are comparable to those commercialized species (Voon and Kueh, 1999). However, the potential value and benefits properties of many other underutilized vegetables are yet to be proven since most of the previous studies only focused on the ethnobotanical knowledge while chemical analyses upon these edible plants are rare. (Ng *et al.*, 2012).

In Sabah, various types of native vegetables grow wildly in the tropical rainforest and this types of plant ususally known as "underutilized" due to it unknown features and are familiar only among the local community. Therefore, this phytochemical study and the antimicrobial activity of the *P. geminiflora* is initiate to make well known the potential values of the plants which can give lots of benefits to local communities and consumers.

1.2 Justification of study

Many studies of the effect of extraction solvents on phytochemical contents and antimicrobial activity have been done on various plants such as fruits, vegetables and medicinal plants but only a few study done on underutilized vegetables of Sabah particularly the *P. geminiflora*. The study of this underutilized vegetable need to be done because of its potential values on giving health benefits to the consumer.

This study will revealed the total phytochemical contents and the antimicrobial activity of *P. geminiflora* which will show the nutritional values and the health benefits properties to against any bacteria that can cause disease. Those information will benefits a lots of people such as the future researcher, the local communities where this vegetable is grown and to the consumer as well.

The future researcher might use those information to help in the further study of underutilized vegetables. As lots of future researchers involve in the study of this underutilized vegetables, it will indirectly promoting this vegetable widely not only in Borneo but may be commercialize to Peninsular Malaysia. Thus, it will benefits the local communities especially those involve in cultivating and selling this vegetable in increasing their daily income.

In spite of everything, the purpose of this study is to promote this potential underutilized vegetable and make it well known not only to the local communities but to the outside communities that live in urban area. The result of this study will give benefits to the consumers on the exact nutritional values and the health benefits properties if it will be included in their diets intake.

1.3 Objectives

The objectives of this study were:

I. To test the effect of different extraction solvents such as methanol, ethanol and hexane on the quantification of phytochemical contents of *P. geminiflora*.

II. To test the effect of different extraction solvents such as methanol, ethanol and hexane on the antimicrobial activity of *P. geminiflora*.

1.4 Hypothesis

Hypothesis for objective is as follow:

H_0: There is no significance difference between the different extraction solvents such as methanol, ethanol and hexane on the quantification of phytochemical contents of *P. geminiflora*.

H_a: There is significance difference between the extraction solvents such as aqueous, methanol and ethanol on the quantification of phytochemical contents of *P.geminiflora*.

CHAPTER 2

LITERATURE REVIEW

2.1 *Plectocomiopsis geminiflora*

Plectocomiopsis geminiflora is a rattan of disturbed forests and of primary forests where light gaps form. It has a natural distribution within Southeast Asia, namely Southern Burma, Southern Thailand, the Malay Peninsula, Sumatra, and Borneo (Dransfield, 1982). It belongs to the large palm subfamily Calamoideae which contains the rattans and the species is variable and ecotypes are found in their respective biogeographical regions (Tan *et al.*, 2011).

2.1.1 Plant morphology

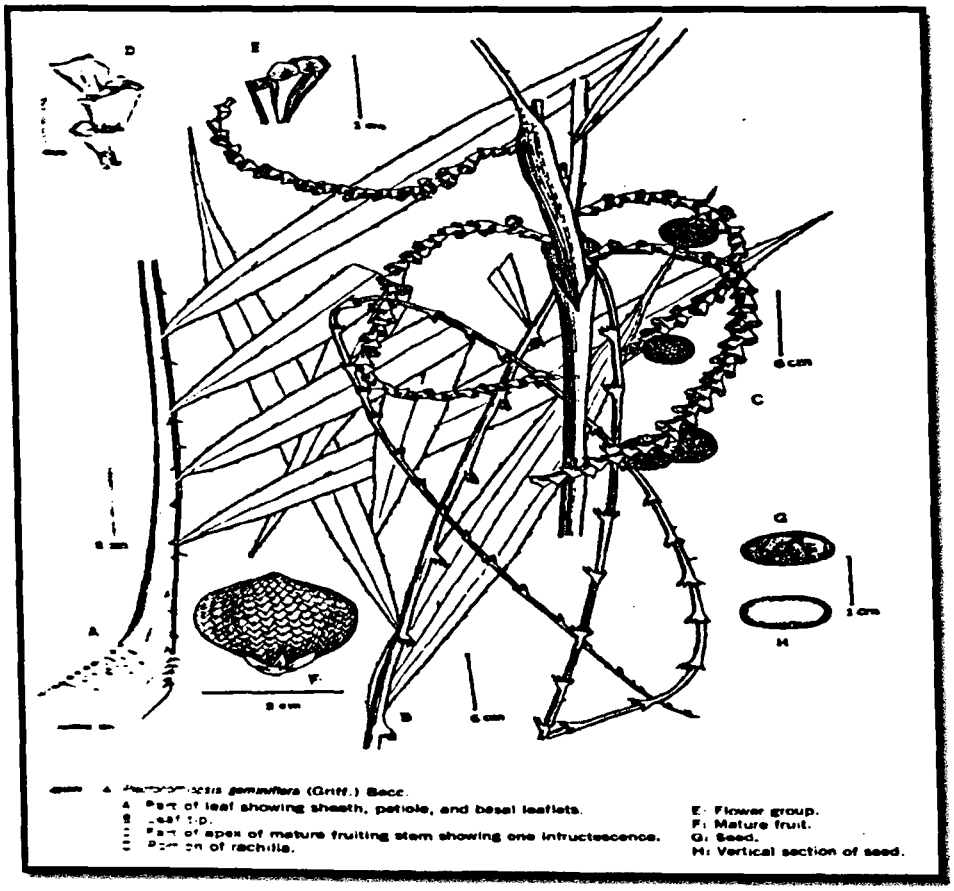

Figure 1: *Plectocomiopsis geminiflora* (Forest Department, Sabah, 1984).

P. geminiflora is a large, spiny, clustering, dioecious, hapaxanthic palm. Hapaxanthly means the stem bearing the inflorescences dies after it has finished flowering. The sheath (part A as shown in Figure 2.1) bears golden-yellow spines up to 1-2 cm long that are variously arranged or sometimes neatly arranged in short rows. The ocrea, the extension of the leaf sheath, is present on young parts but disintegrates and is present as a scar in the old sheath. There are about 30 leaflets, regularly arranged on each side of the leaf rachis and the largest can measure up to 40x4 cm. The leaflets are shiny green, have the same colour on upper and lower surfaces and characteristically bear short bristles on the margin and long golden bristles to 1.5 cm along the mid-nerve on the adaxial surface. None of the cluster were found in the reproductive state. The inflorescences have a main axis that is about 40 cm long with first order branches that reach 30 cm long. The prophyll and the subsequent bracts are tubular and both covered with fine golden scaly hairs. Both male and female flowers are small (about 5 mm long by 2 mm wide). Fruits are spherical to about 3 x 3 cm, with about 32 – 37 vertical rows of chestnut brown scale (Tan *et al.*, 2011).

2.2 Phytochemicals

Phytochemicals are a large group of plant-derived compounds hypothesized to be responsible for much of the disease protection conferred from diets high in fruits, vegetables, beans, cereals and plant-based beverages such as tea and wine (Arts and Hollman, 2005). There are more than thousand known phytochemicals and some of the well-known phytochemicals are phenolic, flavonoid and alkaloid compound.

2.2.1 Phenolic

Phenolic acids are secondary metabolites extensively spread throughout the plant kingdom. Phenolic compounds confer unique taste, flavour, and health-promoting properties found in vegetables and fruits (Barberan and Espin, 2001). Therefore, increasing the phenolic content in these plants can enhance their quality. Phenolic compounds are crucial for plants growth and reproduction, and are produced as a response to environmental factors (light, chilling, pollution etc.) and to defend injured plants (Valentine *et al.*, 2003). Previous research by Zabidah *et al.*, (2014) revealed that total phenolic content of *P. geminiflora* was 2504.88 mg GAE/100 g dw.

2.2.2 Flavonoids

Flavonoids are secondary metabolites of plants with polyphenolic structure. They are synthesized by the polypropanoid pathway and the startup component is phenylalanine molecule. The biological effects of these compounds vary. Most flavonoid compounds which are often accumulated in the vacuoles of plant cells are glycosides. Glycosides can either

be O- or C- linked. The variant of flavonoid glycosides are based on the number of positions on the flavonoid for glycosylation, the level of glycosylation and the number of types of sugars involved in glycosylation. Furthermore, of the several hundred aglycones isolated from plants, only eight are distributed widely (Seigler, 1998) and the eight most common flavonoid nuclei are kaempferol, quercetin, rutin, catechin, epicatechin, myricetin, anthocyanidins and luteolin. Flavonoids are well known for their antioxidant activity. Study that has been carried out by Zabidah *et al.,* (2014) quantified that total flavonoid content of *P. geminiflora* was 6.85 mg RE/100 g dw.

2.2.3 Alkaloid

Alkaloid has been used in extracted form for a very long time as poison; for example narcotic and medicine (Kutchan, 1995). Nitrogen presence in alkaloid is toxic to human body and has physiological activity. It is generally colourless but a few with colour like berberine and betaine; bitter and it is an optically active compound. Alkaloid is in liquid form in room temperature (Harbone, 1984). Alkaloid is soluble in non-polar solvents like chloroform or ether (Ali, 2002). Normally, to testify the presence of alkaloid, sample is tested with six different reagents; Dragendroff's Mayer's, Wagner's, Hager's, Tannic acid test and Ammonia Reineckate Test (Ali, 2002). All six tests must be performed in conformational of the alkaloid substance but it's depend on the availability of the reagents in the lab.

2.3 Antimicrobial activity

The use of plant extracts and phytochemicals both with known antimicrobial properties is of great significance, in the past few years a number of investigations have been conducted worldwide to prove antimicrobial activities from medicinal plants (Alonso-Paz *et al.,* 1995). An antimicrobial is a compound that kills or inhibits the growth of microbes such as bacteria. Such a compound is said to have antibacterial activity (Jagessar *et al.*, 2008). Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are a part of the essential oils (Jansen *et al.*, 1987). There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Rojas *et al.*, 2003). Therefore this study is carried out to know the potential of the nutritive value it may contain. Four bacteria has been chosen to use in this study which were *Eschoria coli (E. coli), Bacillus cereus (B. cereus), Salmonella typhii (S. typhii) and Staphylococcus aureus (S. aureus).*

2.4 Extraction method

Extraction is a very common laboratory procedure used to isolate or purifying a product. The variations in different extraction methods that will affect quantity and secondary metabolites composition of an extract depend upon type of extraction, time of extraction, temperature, nature of solvent, solvent concentration and polarity (Pandey and Tripathi, 2013). Variation in extraction methods usually depends upon length of the extraction period, solvent used, pH of the solvent, temperature, particle size of the plant tissues and the solvent-to-sample ratio (Pandey and Tripathi, 2013). In this study, extraction method used was shaking using waterbath shaker. This simple widely used procedure involves leaving the pulverized plant to soak in a suitable solvent in a closed container .This method is performed at room temperature by mixing the ground drug with the solvent (drug solvent ratio : 1:5 or 1:10) and leaving the mixture for several days with occasional shaking. The extract is then repeated from the plant particles by straining . The process is repeated for once or twice with fresh solvent .Finally the last residue of extract is pressed out of the plant particles using a mechanical press or a centrifuge. The method is suitable for both initial and bulk extraction. The main disadvantage of this method is that the process can be quite time-consuming, taking from a few hours up to several weeks.

2.5 Extraction solvents

For successful determination of biologically active compounds from plants material is largely reliant on the type of solvent used in the extraction procedure. A property of a good solvent in plant extractions includes low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action and inability to cause the extract to complex or dissociate (Pandey and Tripathi, 2013). The factors of affecting the choice of solvent are, the quantity of phytochemical to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compound extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process and potential health hazard of the extractants (Pandey and Tripathi, 2013). The choice of solvent influenced by what is intended with the extract. Since the end product will contain traces of residual solvent, the solvent should be nontoxic and should not interfere with the bioassay (Pandey and Tripathi, 2013). The choice will also depend on the targeted compounds to be extracted. In this study, distilled water, 70% ethanol and 70% methanol were used as extraction solvents.

2.5.1 Distilled water

Distilled water is a colourless, limpid liquid, without odor or taste, and of neutral reaction (Sulz, 1888). It is almost completely pure and does not contain any impurities. In research project, distilled water is suitable for conducting experiments (Boshoff, 2005).

Water is called the "universal solvent" because it dissolves more substances than any other liquid. This means that wherever water goes, either through the ground or through our bodies, it takes along valuable chemicals, minerals, and nutrients (Eloff, 1998).

Water has a high specific heat index. This means that water can absorb a lot of heat before it begins to get hot. This is why water is valuable to industries and in your car's radiator as a coolant. The high specific heat index of water also helps regulate the rate at which air changes temperature, which is why the temperature change between seasons is gradual rather than sudden, especially near the oceans.

Water has a very high surface tension. In other words, water is sticky and elastic, and tends to clump together in drops rather than spread out in a thin film. Surface tension is responsible for capillary action, which allows water (and its dissolved substances) to move through the roots of plants and through the tiny blood vessels in our bodies.

A lot of water is used for cooling purposes in power plants that generate electricity. They need cool water to start with, and they generally release warmer water back to the environment. The temperature of the released water can affect downstream habitats. Temperature also can affect the ability of water to hold oxygen as well as the ability of organisms to resist certain pollutants.

In previous study by Pandey and Tripathi (2013), it revealed that water used as extraction solvent can extract active components such as anthocyanins, starches, tannins, saponins, terpenoids, polypeptides and lectins.

2.5.2 Ethanol

Ethanol is a clear, colourless liquid rapidly absorbed from the gastrointestinal tract and distributed throughout the body. It has bactericidal activity and is used often as a topical disinfectant. It is widely used as a solvent and preservative in pharmaceutical preparations.

Ethanol is a monohydric primary alcohol. It melts at - 117.3°C and boils at 78.5°C. It is miscible (i.e., mixes without separation) with water in all proportions and is separated from water only with difficulty; ethanol that is completely free of water is called absolute ethanol. Ethanol forms a constant-boiling mixture, or azeotrope, with water that contains 95%

ethanol and 5% water and that boils at 78.15°C; since the boiling point of this binary azeotrope is below that of pure ethanol, absolute ethanol cannot be obtained by simple distillation.

In previous study by Pandey and Tripathi (2013), it revealed that ethanol used as extraction solvent can extract active components such as tannins, polyphenols, polyacetylenes, flavonols, flavonols, terpenoids, sterols and alkaloids.

2.5.3 Methanol

Methanol, also known as methyl alcohol, wood alcohol, wood naphtha or wood spirits, is a chemical with formula CH_3OH (often abbreviated MeOH). It is the simplest alcohol, and is a light, volatile, colorless, flammable, and liquid with distinctive odor that is very similar to but slightly sweeter than ethanol (drinking alcohol). At room temperature it is a polar liquid.

Methanol is produced naturally in the anaerobic metabolism of many varieties of bacteria, and is ubiquitous in the environment. As a result, there is a small fraction of methanol vapor in the atmosphere. Over the course of several days, atmospheric methanol is oxidized with the help of sunlight to carbon dioxide and water.

In previous study by Pandey and Tripathi (2013), it revealed that methanol used as extraction solvent can extract active components such as anthocyanin, terpenoids, saponins, tannins, xanthoxyllines, totarol, quassinoids, lactones, flavones, phenones and polyphenols.

CHAPTER 3

METHODOLOGY

3.1 Place and duration of the study

This study was conducted in the laboratory located at the Faculty of Sustainable Agriculture, Sandakan, Universiti Malaysia Sabah. This study had taken about three months' time, starting on July 2015 until September 2015 which including sample collection and phytochemical analysis.

3.2 Preparation of plant sample

P. geminiflora was purchase from Pasar Tamu in Keningau, Sabah, Malaysia. About 3 kg of the plant sample has been collected and brought back to Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, Malaysia and store in a freezer (-20 °C). The peels of the plant sample were removed, and the edible parts were cleaned and chopped into small pieces of same size. The sample was placed neatly on aluminum tray and put in the oven at 40°C for 4 days. The dried plant sample was then grinded to a fine powder using blender and the powder form of plant sample was kept in an airtight plastic bag and stored in -4°C refrigerator for further uses (Noojaree, 2005).

3.2 Phytochemical extraction

In this study, the extraction method was according to Liu *et al.,* (2008) with slight modification. 30 grams of powder extract was mixed with 250ml of extraction solvent (distilled water, 70% ethanol and 70% methanol) and gently shaken at room temperature using waterbath shaker set at 150 rpm and 30°C for 24 hours. After that, the sample extracts were filtered through Whatman paper number 1, and the filtrate was evaporated on a rotary evaporator under vacuum at a temperature of 50°C to dryness and then it were kept in Bijou bottle and stored in -4°C refrigerator for other uses.

3.4 Qualitative analysis of phytochemical contents

Distilled water, ethanol and methanol extracts of *P. geminiflora* were tested for the presence of various phytochemicals contents by using following standard methods:

3.4.1 Test for saponins

One ml of crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins (Yadav and Agarwala, 2011).

3.4.2 Test for glycoside

One ml of crude extract was mixed with 2 ml of chloroform. Then 2 ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring (Yadav and Agarwala, 2011).

3.4.3 Test for flavonoids

One ml of crude extract was mixed with 2 ml of 2% NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids (Yadav and Agarwala, 2011).

3.4.4 Test for steroid

One ml of crude extract was mixed with 2 ml of chloroform and concentrated H_2SO_4 was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids (Yadav and Agarwala, 2011).

3.4.5 Test for quinones

One ml of crude extract was treated with concentrated HCl and the formation of yellow colour indicated the presence of quinones (Lalitha *et al.*, 2012).

3.4.6 Test for phenols

One ml of crude extract was treated with 5% $FeCl_3$ and the formation of black colour indicated the presence of phenols (Lalitha *et al.*, 2012).

3.4.7 Test for anthocyanin

One ml of crude extract was treated with 2M NaOH and the formation of blue green colour indicated the presence of anthocyanin (Lalitha *et al.*, 2012).

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