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Original Article

Phytochemical screening of *Euphorbia hirta* linn leaf extracts

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ABSTRACT

Euphorbia hirta belongs to the family Euphorbiaceae is well known for its medicinal properties and widely used worldwide. *Euphorbia hirta* leaves was investigated for its Ash value, extractive value, total moisture content and fluorescence analysis. Further, the present study aimed to identify the various phytochemical constituents present in the three different extracts by using standard methods of phytochemical screening. Powdered plant material contained 8.90% Ash value, 7.0% w/w water soluble extract, 14.85% w/w ethanol soluble extract, 9.71% w/w methanol soluble extract, 9.84% w/w moisture content and fluorescence to some treatments. The chemical constituents of the ethanolic and methanolic leaf extracts of the plant were relatively similar in the presence of proteins, fats and oils, gums and mucilages as primary metabolites and alkaloids, flavonoids, terpenoids, tannin, phenol, steroid, glycoside, saponin, coumarin as secondary plant metabolites. Amino acids, carbohydrates and anthraquinones did not detected. In contrast, aqueous extract appeared to posses the constituents such as carbohydrates, proteins, amino acid, tannin, phenol, steroid, saponin and anthraquinone with a negative result to alkaloids, flavonoids, terpenoids, glycosides, fat and oils, coumarin, gums and mucilages. In conclusion, the reported results support the leaf extracts of *Euphorbia hirta* to exhibit a wide spectrum of pharmacological activities and promisingly used as traditional medicine.

Key Words: *Euphorbia hirta*, physio-chemical analysis, phytochemical screening, aqueous extract, maceration, ethanol extract, methanol extract.

INTRODUCTION

Phytochemistry or Plant chemistry (the Greek word "Phyto" meaning plant) is the branch of chemistry, deals with chemical nature of the plant or plant products (chemistry of natural products). Phytotherapy acts as a source of treating and improving certain diseases by using the beneficial effects of medicinal plants. Phytochemicals are the bioactive, natural chemical compounds, found in plants. The plant contains a wide variety of chemical compounds and they are broadly classified into two types, primary and secondary metabolites. Primary metabolites are mainly involved in the primary metabolic processes such as building and maintaining plant cell [1]. Most of the primary metabolites are universal in occurrence. Primary metabolite includes carbohydrates, lipids, proteins, amino acids and chlorophyll, etc. Secondary metabolites includes alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds [2,3]. Secondary metabolites have some valuable biological properties like antioxidant activity, antimicrobial activity, antibacterial, antifungal, anticonstipative, spasmolytic, anticancer, modulation of detoxification enzymes, immune system stimulation, decreased platelet aggregation and hormone metabolism modulation, etc. The secondary metabolites are naturally synthesized in all parts of the plant body especially bark, leaves, stem, root, flower, fruits, seeds, etc [4]. Plants of different species or of same species grown in different location or harvested at a different time would have different chemical constituents. Different parts such as leaves, bark, seeds, roots, flowers and pods of plants also have different quality and quantity of active constituent [5]. In

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facts, the information on the distribution of active compounds or active principles is lacking. In turn, these phytochemicals protects the plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack. It also contribute the plant's colour, aroma and flavor. Medicinal plants serve as an important source of bioactive molecules for novel therapeutic agents [6]. Now days, the novel therapeutic agent used against many diseases. Isolated bioactive molecules are utilized in the synthesis of new drugs. Hence, Extraction plays an important step in the itinerary of phytochemical processing for the discovery and isolation of bioactive constituents from plant materials. Extracts prepared using different extraction techniques is reported to have variations in biological activities. Therefore, it is necessary to select the suitable solvent and suitable extraction method as an important step in standardization of herbal products.

METHODS AND MATERIALS

Chemicals: All the chemicals were of analytical grade and were obtained from Merck India Limited and Laba Chemie Limited, India.

Authentication of plant material: The whole plant parts of *Euphorbia hirta* Linn were collected from several habitats at Vellore district, region, Tamil Nadu and were authenticated by Dr. P. Jayaraman, Retd. Professor, Presidency college, Chennai. The voucher specimen was preserved in the Department of Biochemistry, Adhiparasakthi College of Arts and Science, Kalavai, for further reference. The certificate of authentication is given in Annexure.

Collection of plant material: The leaves were separated from the whole plants and collected. The leaves were initially washed with distilled water to remove debris and dust particles, dried on paper towels at room temperature $(37\pm1)^0$ C. The leaves were avoided from exposure to sunlight to prevent the loss of active components. Dried leaves were packed in polyethylene bags and stored at -18^0C until used. In the laboratory, after drying, the leaves were subjected to size reduction in a mechanical grinder, followed by a blender, to get coarse or fine powder. The coarse powder was then passed through sieve no. 40 mm to get desired particle size and stored in a well – closed plastic containers at room temperature till required for use.

Determination of Physio-Chemical Constant: Physic-chemical values such as the ash values, extractive values and percentage of loss on drying were carried out as per the standard method described in Indian Pharmacopoeia (1996).[7]

Determination of Ash values [8,9]: Crude drugs quality and purity was determined by ash values. Sodium, potassium, magnesium, carbonates of calcium salts, phosphates and silicates constitutes the total ash values. Powdered plant materials were subjected to total ash, acid insoluble ash and water soluble ash.

Determination of Total Ash [10]: Coarsely powdered leaf material of about 2 to 3gms was weighed and transferred to a pre-weighed silica crucible. In the crucible, powdered drug was spread evenly as fine layer and incinerated by gradually increasing the temperature not exceeding 450^0c until colourless, indicates the carbon free. After incineration completion, silica crucible was cooled in a desiccator and weighed. The same procedure was repeated to get a constant weight. The percentage of total ash was determined with reference to the shade dried plant material.

Acid –insoluble ash: The ash obtained from the above method was boiled for 5-10min with 25ml of dilute HCl. The insoluble ash was collected in an ashless filter paper or a Gooch crucible. The crucible or filter paper was washed with hot water. The acid insoluble ash was transferred to a pre weighed silica crucible. This was repeated until constant weight obtained. The acid insoluble ash percentage was calculated with reference to the air –dried drug.

Water soluble ash value: The ash obtained in the determination of total ash was boiled for about 5-10min in 25ml of water. The insoluble matter was collected in a silica crucible, washed with hot water. The insoluble ash so obtained was transferred to a pre-weighed silica crucible. The silica crucible was heated gradually to a temperature of 450^0c for 15 min to get a constant weight. The crucible was cooled and weighed. The percentage of water soluble ash was calculated by subtracting weight of insoluble matter from the weight of total ash.

Moisture content (loss on drying) (LOD): 2gms of powder drug was accurately weighed and taken in a porcelain dish. The dish was kept in a hot air oven maintained at a temperature 110^0c for 4 hours until a constant stable weight was recorded. The procedure was repeated. The dish was cooled in a desiccator at room temperature and weighed.

Fluorescent analysis: The fluorescence behavior of the leaf powder of *Euphorbia hirta* after treatment with various chemical reagents like sulphuric acid, hydrochloric acid, nitric acid, sodium hydroxide, ferric chloride, iodine solution, picric acid, potassium hydroxide, acetic acid,

ethanol, methanol, lead acetate solution and ammonia was determined according to the methods of Chase and Pratt 1949 [11]. All the treatments were subjected to fluorescence analysis and examined under both day light and UV light (254 nm and 365 nm).

Preparation of the Extract: The term extraction involves seperation of therapeutically required portions of the plant materials from the inactive components by using successive solvents (Menstruum). The extraction and isolation of secondary metabolites from plants is an growing interest.

Extraction of crude drug: The powdered plant material was extracted by two methods.

***Extraction by cold maceration or preparation of aqueous extract*:** About 100gm of dry powdered plant material was subjected to cold maceration with distilled water in a 1 liter conical flask. The flask was plugged with an adsorbent cotton and kept aside for about 24 h at room temperature with frequent agitation or occasional shaking. After the completion of maceration, the marc was placed in a muslin cloth and filtered. The filtrate was again filtered using whatmann No:1 filter paper [12]. This procedure of soaking and filtrations was repeated for two or more times until it becomes colourless and all the extracts were pooled together and then concentrated in vaccum at 40^0C using rotary evaporator to a thick, semisolid pasty mass of dark black colour. The residues thus obtained were stored in a freezer at -70^0 C until ready for use.[13]

Successive extraction by using soxhlet apparatus:
Principle: To prepare various extracts of *Euphorbia hirta* Linn., a successive solvent extraction procedure was adapted. The plant materials were subjected to successive extraction with different solvents, starting from solvent of low polarity to high polarity.
Materials: Dried leaves powder of *Euphorbia hirta* Linn.
Solvents: Methanol, Ethanol.
Procedure: An about 35 gm of powdered drug of the selected medicinal plant was subjected to extraction by using methanol as solvent in a soxhlet apparatus. The extraction was continued until the solvent in the timble becomes clear or colourless. Then the heating was stopped and the mixture from distillation flask was collected and cooled. Then this mixture was filtered and concentrated by using evaporator at room temperature. The extract was dried at room temperature and stored in amber coloured glass jar in a freezer or desiccator and was used for further experiments [13]. The marc obtained after extraction was removed, dried and recharged, extracted with ethanol (95%) solvent of successively higher polarity to collect ethanolic extract.

Determination of extractive value: Extractive values are used to evaluate the nature of phytochemicals present in the crude drugs. The extractive yield is a measure of the solvent's efficiency to extract specific components from the original material and it was defined as the amount of extract recovered in mgs compared with the initial amount of whole plant. It is presented in percentage and was determined for each techniques tested.

Qualitative phytochemical screening of various plant extracts: Methanol and ethanol extract obtained by successive solvent extraction method and aqueous extract by cold maceration method. The above three concentrated extracts of the *Euphorbia hirta* Linn were subjected to various standard phytochemical tests procedures to detect the presence or absence of various active phytoconstituents present in the crude extracts.

RESULT

In the present investigation, *Euphorbia hirta* leaves have been subjected to physio-chemical and phytochemical screening to detect phytochemical constituents. Ash value includes total ash, acid insoluble ash, and water soluble ash. The Ash value of Euphorbia *hirta* leaves were determined and the results are depicted in Table 1.1 and figure 1a. The total ash, acid insoluble ash and water soluble ash values of powdered Euphorbia *hirta* leaf was found to be 8.90, 7.84 and 1.06 % w/w respectively. Percentage yield of the selected successive extracts of *Euphorbia hirta* leaf powder were recorded in Table No.1.1. To found out the percentage yield of individual extracts extractive values were analysed. From the result it was found that, the extractive values were found to be 14.85% w/w with ethanol and 9.71% w/w with methanol. While 7.0 % w/w yield with aqueous extract. The moisture content of powdered *Euphorbia hirta* was shown in table 1.1. The moisture content was found to be 9.84%. The fluorescence analysis of the powdered plant material towards ordinary light and ultraviolet light (long wavelength 366nm and short wavelength 254nm) on treatment with various reagents are shown in Table 1.2. The results indicated the presence of particular phytoconstituents in the plant powder by fluorescence and its presence was later confirmed by the phytochemical test. Details of various tests performed for the presence or absence of phytoconstituents in the three different extracts as per the standard procedures were summarized in

Table 1.3. The three extracts aqueous, ethanol and methanol of *Euphorbia hirta* leaves were evaluated for the detection of its phytochemical constituents, it includes carbohydrate, amino acid, proteins, alkaloid, flavonoid, terpenoid, tannin, phenol, steroid, glycoside, saponin, coumarin, anthraquinone, volatile oils, gums and mucilage, fat and oils. Results of the preliminary phytochemical analysis carried out on the crude ethanol and methanol extract of *Euphorbia hirta* (leaves) indicated the presence of protein, fats and oils, gums and mucilage and alkaloids, flavonoids, terpenoids, tannin, phenol, steroid, glycoside, saponin, coumarin as secondary plant metabolites. The phytochemical study did not detect any amino acids, carbohydrates and anthraquinones. The aqueous extract showed the positive result for carbohydrate, protein, amino acid as primary plant metabolites and Tannin, phenol, steroid, saponin, anthraquinone as secondary plant metabolites. Alkaloids, flavonoids, terpenoid, glycosides, fat and oils, coumarin, gums and mucilages were not detected in aqueous extract.

DISCUSSION

Residue that remains after the complete incineration of the plant material is termed as ash value. The ash values are particularly helpful in determining the quality, purity of crude drug and also gives an idea about the earthy matter or the inorganic composition like silica, clay and other impurities (adulteration) present along with the powdered form by following the standard procedure [28]. In the present study, the total Ash value of *Euphorbia hirta* indicates the amount of minerals like carbonate, oxalate, silicate and the earthy materials present in the plant material and its value was calculated to be 8.90 % (w/w). Interestingly, the plant sample has more acid insoluble ash (ash insoluble in dilute HCl) value of 7.84% w/w than water insoluble ash 1.06% w/w respectively. The water soluble ash is used to detect the presence of material soluble in water. Similar reports were also made by previous researchers in different plants such as *Evemurus himalaicus Baker* [29], *Cardiospermum halicacabum* Linn.,[30], *Nigella sativa* Linn.,[31], *Berginia ligulata* and *Ammania buccifera* Linn.,[32], *Persea macrantha* (Nees) *Kosterm* [33], *Leucas cephalotes* Spreng [34] and *Evovulus nammularius* [35]. The water soluble extract indicates the presence of sugars, acids and inorganic compounds and the value was found to be 7.0% w/w. The ethanol soluble extract was 14.85% (w/w) and methanol soluble extract was 9.71 % w/w. These alcoholic extractive values indicates the presence of polar constituents such as phenols, steroids, glycosides, flavonoids, alkaloids and secondary metabolites present in the *Euphorbia hirta* sample. Extractive values are useful to evaluate the nature of the chemical constituents present in them. The Extractive values also estimates specific constituents soluble in a particular solvent. The percentage yield of extract was abundant in ethanol rather than methanol and aqueous. The amount of extracts to a given solvent gives an approximate measure of certain constituents of group of related constituents the drug contains. However, results indicate that relatively more constituents are present in ethanol extract, hence, ethanolic extracts were selected for further studies. These results are in accordance with many workers obtained from the extraction of different plants with different solvents by many workers [36, 37, 38, 39, 40, 41, 42, 43]. The moisture content of the powdered *Euphorbia hirta* leaves material was calculated to be 9.84% as observed in the previous work Pravin Aabsing Theng *et al*., 2014 [44]. The results suggest that the moisture content of *Euphorbia hirta* leaves was found to be very minimal amount as recommended (not more than 14%) and hence can be stored at room temperature without fear of spoilage. It also indicates fewer chances of microbial degradation of the drug during storage because high moisture content can result in the breakdown of important constituents by enzymatic activity and which in turn encourage the growth of yeast and fungi during storage. The powdered leaves of *Euphorbia hirta* exhibited a clear fluorescence behavior by various chemical constituents when observed under visible range in day light, and UV light at two different wavelengths 254nm and 365 nm. Similar fluorescence analysis results was obtained in plants [44, 45, 46, 47]. Preliminary phytochemical screening is an important initial step to find out the phytoconstituents present in the plant extract, which further leads to the isolation of active compounds, responsible for many pharmacological actions. The solvents used were aqueous, ethanol and methanol. In *Euphorbia hirta* leaves, the ethanol and methanol extract detected the presence of proteins, fats and oils, gums and mucilage, alkaloids, flavonoids, terpenoids, tannin, phenol, steroids, glycoside, saponin and coumarin whereas the phytochemical characterization tests revealed the absence of amino acids, carbohydrates and anthraquinones. The aqueous extract shows the presence of carbohydrates, proteins, amino acids, tannin, phenol, steroids, saponin and anthraquinone. In addition, alkaloids, flavonoids, terpenoids, glycosides, fat and oils, coumarin, gums and mucilages were not identified by these methodologies. From the results, it was confirmed that the plant *Euphorbia hirta* has quite a number of chemical constituents [48] which may be responsible for many pharmacological actions and

medicinal properties [49,50]. Alkaloids have been reported to have antimicrobial, analgesics/ narcotics, antileukaemic agents [51]. Flavonoids are a class of phenolic compounds usually found in all plants parts like leaves, seeds, fruits, bark and flowers either in the aglycone form or as glycone derivatives. Therapeutically flavonoids have been employed to possess some biological actions and pharmacological effects like antioxidant, anti-inflammatory, anti-cancer, anti-diabetic, anti-atherosclerotic, anti-viral, antiallergic, hepatoprotective, diuretic effect [52, 53, 51, 54], spasmolytic, hypotensive and oestrogenous activities [55].

Structurally saponins consists of non-polar aglycones, coupled with one or more monosaccharide moieties are reported to have numerous properties, which include pharmacological activities such as anti-microbial, anti-inflammatory [56] as well as sweetness and bitterness, foaming and emulsifying properties [57]. In most of the plants, saponins are found to be vulnerable to fungal or bacterial and insect attacks [58]. This property suggests that saponin acts as a chemical barrier against potential pathogens in plants [59].

Tannin a biologically active phytoconstituent have been found to possess spasmolytic activity, free radical scavenger property and antioxidant activity as pharmacological activities. [60, 61, 62].

Plants rich in phenolic contents have been reported to have effective beneficial effects such as antioxidant activity, antimicrobial, anti-inflammatory, antiviral, antimutagenic and chemoprotective effects [63] at lower concentrations. Therefore, saponins are widely used in traditional medicine as treatment for many diseases [64].

Therapeutically, terpenoids are reported to exhibit antiviral, antibacterial, anti-tumour, antiseptic, diuretic and analgesic activities [65]. Biologically active compounds usually occur in low concentrations in plants. An extraction technique is one which is able to obtain extracts with high yield and hence different extraction techniques reported to have variations in the biological activities. Hence, it is necessary to select the suitable extraction method.

In the present study, two extractions methods are used to evaluate the phytochemical constituents of leaf extracts, maceration and continuous percolation method (Soxhlet extraction). Comparison of these two extraction methods reveled to produce different results, however, there are some qualitative differences exist. Among the different solvent extraction methods successive Soxhlet extraction provides a comparable better results than the maceration. Eventhough, in Soxhlet extraction heat sensitive compounds undergo decomposition it showed a better result. However, thermolabile / thermostable compound are stable and shows a good anti-oxidant, anti-inflammatory properties compared to other technique (maceration). It also showed a significant extraction time and solvent consumption advantages over another method maceration. Overall, continuous percolation method was found to be best in obtaining antioxidant, anti – inflammatory active compounds from the plant powder than the maceration method.

CONCLUSION:

The study revealed the presence of a wide range of phytochemical constituents in leaves extracts of *Euphorbia hirta* Linn. This might help us to validate the ethnomedicinal uses. Now our efforts are concerned with isolation of active compounds from the selected plant *Euphorbia hirta* Linn.

Table No 1.1: DATA SHOWING ASH VALUES, EXTARCTIVE VALUES, MOISTURE CONTENT OF POWDERED LEAVES OF EUPHORBIA *HIRTA.*

S.NO	Physio-Chemical Analysis	Yield (% w/w)
1.	**Ash values** Total Ash	8.90
	Acid insoluble Ash	7.84
	Water soluble Ash	1.06
2.	**Extractive values** Water soluble extract	7.0
	Ethanol soluble extract	14.85
	Methanol soluble extract	9.71
3.	**Moisture content**	9.84

Figure 1a: Ash values of *Euphorbia hirta.*

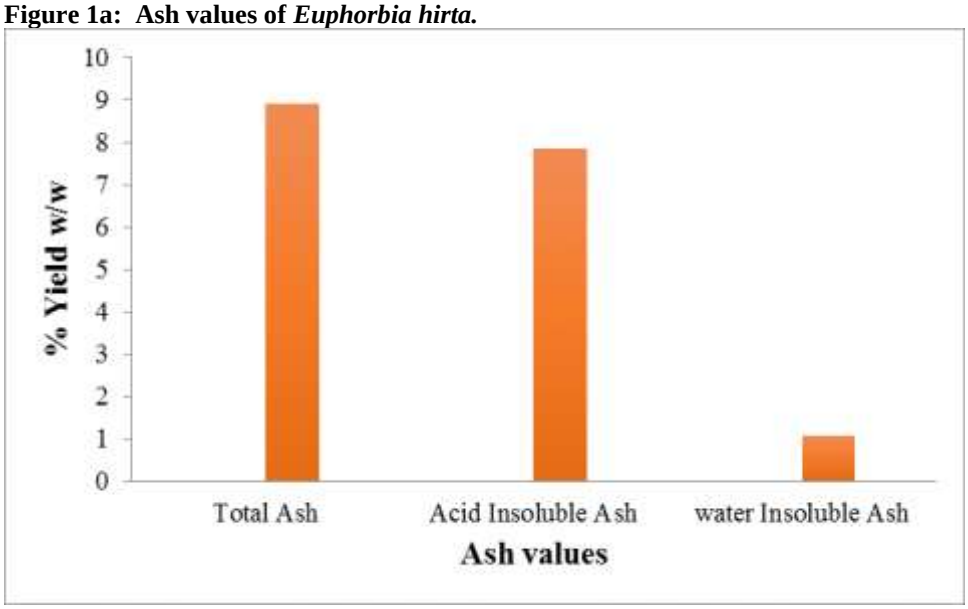

Table 1.2: FLUORESCENCE ANALYSIS OF THE POWDERED LEAVES OF *EUPHORBIA HIRTA* LINN

Experiments	Powdered Drug		
	Fluorescence under Visible/day light	**Fluorescence under short UV (254nm)**	**Fluorescence under Long UV(365nm)**
2.0 grams of powder as such	NF	NF	NF
0.5 g of powder + 5ml of 1M NaOH(Alcoholic)	NF	F	F
0.5 g of powder + 5ml of 1M NaOH(Aqueous)	NF	F	F
0.5 g of powder + 5 ml of 50% HCL	NF	NF	NF
0.5 g of powder + 5ml of 50% HNO3	NF	NF	NF
0.5 g of powder + 5ml of H2SO4	NF	NF	NF
0.5 g of powder + 5 ml of 5% Fecl3	NF	NF	NF
0.5 g of powder + 5 ml of 5% I2 solution	NF	NF	F
0.5 g of powder + 5 ml of picric acid	NF	F	NF
0.5 g of powder + 5 ml of 1N HCL (Alcoholic)	NF	F	F
0.5 g of powder + 5 ml of 1N HCL (Aqueous)	NF	F	NF
0.5 g of powder + 5 ml of 1N HNO3 (Alcoholic)	NF	F	F
0.5 g of powder + 5 ml of 1N HNO3 (Aqueous)	NF	NF	NF
0.5 g of powder + 5 ml of KOH	NF	F	F
0.5 g of powder + 5 ml of Acetic acid	NF	NF	F
0.5 g of powder + 5 ml of water	NF	NF	F
0.5 g of powder + 5 ml of Ammonia	NF	F	F
0.5 g of powder + 5 ml of Ethanol	NF	F	F
0.5 g of powder + 5 ml of Methanol	NF	F	F
0.5 g of powder + 2.5 ml of 40% NaOH + 2.5 ml of Lead Acetate Solution	NF	F	NF
0.5 g of powder + 2.5 ml of HNO3 + 2.5 ml of Ammonia	NF	F	F

Table 1.3: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF *EUPHORBIA HIRTA* LINN. LEAVES EXTRACT.

S.NO	Phytoconstituents	Aqueous	Ethanol	Methanol
1.	**Carbohydrates**			
	a. Molisch test	+	-	-
	b. Anthrone test	+	-	-
	c. Fehlings test	+	-	-
	d. Benedicts test	+	-	-
	e. Barfoed's test	+	-	-
2.	**Proteins and Amino Acids**			
	a. Biuret test	+	-	-
	b. Millon's test	+	-	-
	c. Ninhydrin test	+	-	-
3.	**Alkaloids**			
	a. Mayer's test	-	+	+
	b. Wagner's test	-	+	+
	c. Hager's test	-	+	+
	d. Dragendroff's test	-	+	+
4.	**Flavanoid**			
	a. Shinoda test	-	+	+
	b. Alkaline test	-	+	+
	c. Zinc hydrochloride test	-	+	+
	d. Ferric chloride test	-	+	+
5.	**Saponins**			
	a. Foam or froth test	+	+	+
	b. Haemolytic test	+	+	+
6.	**Tannins and Phenolic compounds**			
	a. Vanillin hydrochloride test	+	+	+
	b. Phenazone test	+	+	+
	c. Ferric chloride test	+	+	+
	d. Lead acetate test	+	+	+
7.	**Steroids**			
	a. Salkowski's reaction	+	+	+
	b. Libermann-Burchard's test	+	+	+
	c. Hesse's reaction	+	+	+
	d. Sulfur –powder test	+	+	+
8.	**Terpenoid**			
	a. Salkowski's reaction	-	+	+
	b. Libermann-Burchard's test	-	+	+
	c. Brieskorn and Brinar test	-	+	+
	d. Ischugajju test	-	+	+
	e. Noller's test	-	+	+
9.	**Glycosides**	-	+	+
	1. Anthraquinone glycosides			
	a. Borntrager's test	+	-	-
	b. Modified Borntrager's test	+	-	-
	c. Coumarin glycosides	-	+	+
10.	**Fat & oils**			
	a. Soap test	-	+	+
	b. Spot test	-	+	+
	c. Saponification test	-	+	+
11.	**Gums and Mucilages**	-	+	+

\+ - Presence of compounds

\- -Absence of compounds.

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