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PHARMACOLOGICAL SCREENING OF MANIKARA ZAPOTA FRESH FLOWER BUDS OF DIFFERENT POLAR AND NON POLAR SOLVENTS IN LUNAWADA WILD REGION DIST. MAHISAGAR GUJARAT

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ABSTRACT

Manikara zapota (sapodilla) is a fruit and nut plant that is important to the tribal people who live in tropical deciduous forests in western and central India. The fresh flower buds of the Manilkara zapota, a sapodilla or chiku plant employed in the study, were subjected to a qualitative phytochemical examination. Solvents like acetone, ethyl acetate, and toluene were used to identify flavonoids, alkaloids, proteins, phenols, glycosides, tannins, and steroids. A phytochemical screening was used for the analysis. Thus the present work was aimed to find out phytochemicals present in *Manilkara zapota* through, proximate, histochemical, fluorescence, separation and identification by using the thin layer and column chromatography, characterizing the identified phyto-constituents using analytical techniques such as UV-Visible spectra, FT-IR and Qualitative analysis.

KEYWORDS: Pharmacological Screening, fresh flower buds, Manikara zapota (Sapodilla), Flavonoid, alkoloids, proteins, phenols, glycosides, Tannin.

INTRODUCTION

Manikara zapota (sapodilla) is a fruit and nut plant that is important to the tribal people who live in tropical deciduous forests in westernand central India. The fresh flower buds of the Manilkara hexandra, a sapodilla or chiku plant employed in the study, were subjected to a qualitative phyto chemical examination. Solvent like ethylcetateacet one and toluene were used to identify flavonoids, alkaloids, proteins, phenols, glycosides, tannins, and steroids. A phyto chemical screening was used for the analysis.[1] As a result, the effects of medicines have weakened and become neutral, which has increased drug use and a propensity to employ harsher and never-used chemicals for mulations. An additional issue with chemical medicine use is the rise in side effects, which might result in diseases that are more

deadly than the original condition.[2] Plants contain potent and valuable chemicals such as phenols, phenolic derivatives, quinones, flavones, flavonoids, tannins, and coumarins. Terpenoids and so on essential oils, alkaloids, lectin, and polypeptides.[3] The majority of Munilkara Zapota's common names, such as "Sapodilla," "chiku," and "chicozypote," are derived from Spanish and mean "little Sapote".[4] Other common names in English are bully tree, soapapple tree, Sawo, and marmalade plum.[5] Compounds extracted from the leaves have antidiabetic, antioxidant, and hypocholesterolemic (cholesterol-lowering) properties in rats.[6]

These phytochemicals, which are part of a large and varied group of chemical compounds, also are responsible for the color, flavor, and odor of plant foods, such as blueberries dark hue, broccoli's bitter taste, and garlic's pungent odor. Thousands of phytochemicals have been identified, and researchers speculate that there are likely many more they haven't yet discovered in the foods we eat. Though the broadest groups of phytochemicals, such as flavonoids, isoflavones, or anthocyanidins, often are referred to as a homogenous group, the individual compounds within each group have different chemical structures, are metabolized differently by the body, and may have different health effects.[7] Research strongly suggests that consuming foods rich in phytochemicals provide health benefits, but not enough information exists to make specific recommendations for phytochemical intake.[8] Proximate and nutrient analyses of edible plant and vegetables play a crucial role in assessing their nutritional significance.[9] In general, 22 nutrients are present in each human body for their requirements such as Fe, Ca, Mg, K, and Zn.[10] The antimicrobial activities have shown that the higher plants represent a potential source of novel antibiotic prototypes.[11] This has forced scientist to search for new antimicrobial substances from various sources like the medicinal plants.[12] The stem bark of *Manilkara hexandra* was used to cure gastro intestinal problem, burning sensation, febrifuge, odontopathy, hallucination, dyspepsia, anthelmintic, astringent and tonic.[13]

MATERIAL AND METHOD

Plant material

Fresh Manilkara zapota fresh flower buds were gathered from the Lunawada wild region in the Mahisagar district. The gathered plant matter was unrestricted. The plant's fresh flower buds were dried in the shade prior to flowering, ground into a powder, and then tested using phytochemical analysis in a Soxhlet apparatus with AR grade solvents.

Preparation of the Plant Extract

All the chemicals (AR) were acquired from Sigma Aldrich. The powdered fresh flower buds material was defatted with ethyl acetate, acetone and toluene s by cold percolation method for 3 days. This solution was extracted by utilizing Whatman No.1. filter paper and stored in refrigerator at 4°C.

RESULT AND DISCUSSON

Physical Characterizations

Physiochemical Parameters

The physiochemical factors were analyzed by using WHO guidelines and Indian Pharmacopeia for herbal ingredients.[13] These include total ash content, acid insoluble ash, water soluble ash, moisture content, sulfated ash, and pH.

Total ash - 3g of the sample was weighed accurately in a silica crucible. Then, it was incinerated by using muffle furnace at a temperature 400-500°C. The carbon particle was dried till it turns white in color. The total ash was cooled and weighed.

$$\% \text{ Ash} = \frac{(Weight\ of\ crucible+ash)-Weight\ of\ crucible}{Weight\ of\ sample} \times 100$$

Moisture content

A crucible with a lid was used to weigh 3g of the sample. The sample was cooled with a desiccator after being maintained at 105°C in a hot air oven. The weight difference before and after the drying process was used to determine the subsequent outcomes.

$$\% \text{ Moisture} = \frac{(Weight\ of\ sample+dish\ before\ drying)-(Weight\ of\ sample+dish\ after\ drying)}{Weight\ of\ sample\ taken} \times 100$$

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Acid insoluble ash

Hatman No. 1 filter paper was used to filter the aforementioned ash after it had been heated with 25 milliliters of diluted HCl. This paper was filtered, then dried in a crucible to produce an ash that was insoluble in acid. The crucible's weight was determined both before and after.

Water Soluble ash

In a crucible, the whole ash (A) was cooked for 15 minutes at 450°C with 25 milliliters of distilled water. After filtering this fraction, the insoluble portion (B) was gathered. This insoluble portion was once more dried and weighed. Subtract A from B to determine the amount of water-soluble ash.

Sulfate ash

After 30 minutes of being burned with Con. H2SO4 at 550°C to 650°C, the whole ash was chilled in a desiccator until its weight remained constant.

Dry Matter -Dry matter of flower buds material consists of nutrition excluding the water content. It was obtained from percentage of moisture content.[14]

% Dry matter = 100 – % Moisture

pH- After dissolving the flower buds powder in distilled water, it was brought to a boil for twenty-five minutes. After cooling, the filtrate was gathered. Lastly, a systronic pH meter was used to record the filtrate's pH.

Table : Physiochemical parameters of buds of *Manilkara zeptop.*

S. No.	Proximate Tests	Buds
1	Total ash	5.09±0.12
2	Moisture content	5.25±0.08
3	Acetone soluble ash	0.15±0.54
4	Ethyl acetate soluble ash	1.76±0.12
5	Tolulene insoluble ash	5.47±0.07
6	pH	4.89±0.15

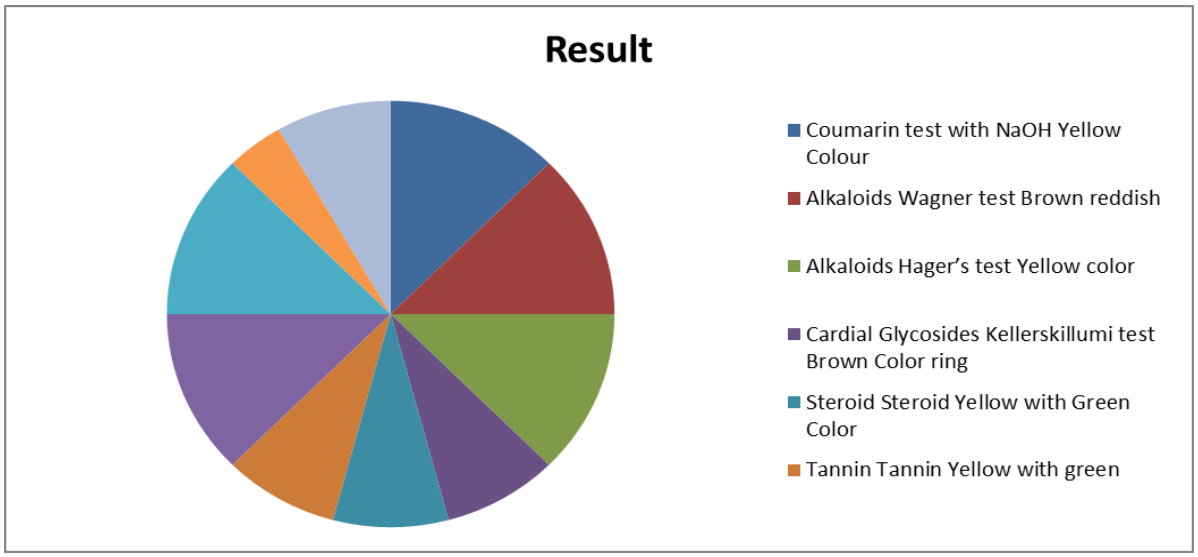
1-Primary phytocamical analysis of flower buds

The flower buds of the Manilkara zapota, a sapodilla or Chiku plant, were used in the study. Flavonoids, alkoloids, proteins, phenols, glycosides, tannins, and steroids were detected using solvents such as ethyl acetate, acetone, and toluene. The analysis was qualitative.

In Ethyl Acetate

Sr. no	Phytochemical Analysis	Test Performance	Observation	Result
1	Coumarin test	10% NaOH	Yellow Color	2
2	Alkaloids	(a)Wagner test	Brown reddish	3
		(b)Hager's test	Yellow color	3
3	Protiens	Xanthoroteic test	No Change	0
4	Cardial Glycosides	Kellers killumi test	Brown Color ring	2
5	Steroid	Liberman (5 drops of acetic anhydride + 5 drops of H_2SO_4	Yellow with Green Color	3
6	Tannin	$FeCl_3$ Solution	No Change	0
7	Amino Acids	Ninhydrin test	No Change	0
8	Anthocyanin	2N HCl + NH_3	No Change	0
9	Emodins	NH4OH + Benzene	No Change	0
10	Phenol	Alcoholic $FeCl_3$ Solution	Bluish black Color	2
11	Flavonid	NH_4OH test	Yellow Color	3
		Alkaliner test	Yellow Colour	3
12	Diterpenes	Copper Acetate test	Green Color	2
13	Saponin	Distil Water	Oil Form	2
14	Phytosterol	Salkowskis test	Golden red indicate	3

0=absent, 1 = average, 2= good, 3= very good

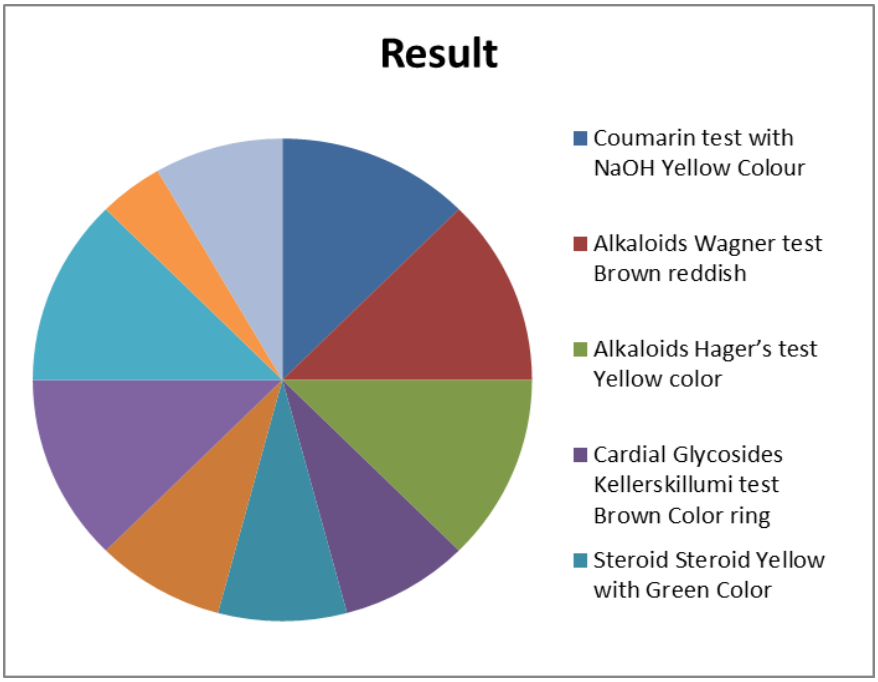


In Acetone Solvent

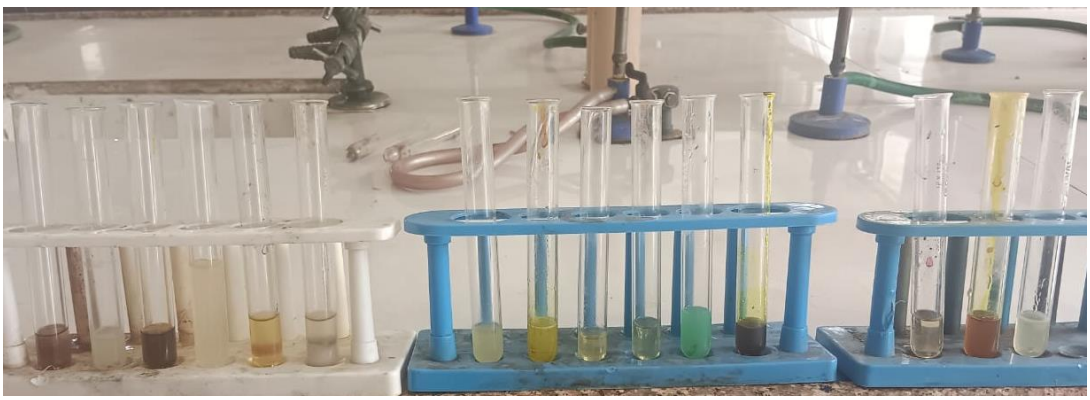
Acetone

Sr. no	Phyto Chemical Analysis	TEST PERFORMANCE	Observation	Result
1	Coumarin test	with NaOH	Yellow Colour	3
2	Alkaloids	Wagner test	Brown reddish	3
		Hager's test	Yellow color	3
3	Cardial Glycosides	Kellerskillumi test	Brown Color ring	2
4	Steroid	Conc.H_2SO_4 + $CHCl_3$	Yellow with Green Color	2
5	Tannin	Lead Acetate	Yellow with green	2
6	Amino Acids	Ninhydrin test	No Change	0
7	Anthocyanin	2N HCl + NH_3	No Change	0
8	Emodins	NH_4OH +Benzene	No Change	0
9	Phenol	Alcohlic $FeCl_3$	Bluish black Color	3
10	Flavonid	NH_4OH test (Alkaliner test)	Yellow Color	3
11	Diterpenes	Copper Acetate test	Green Color	1
12	Saponin	Distil water	Oil Form	2
13	Leuco anthocynine	Isoamyl alcohal	No change	0

0=absent, 1 = average, 2= good, 3= very good

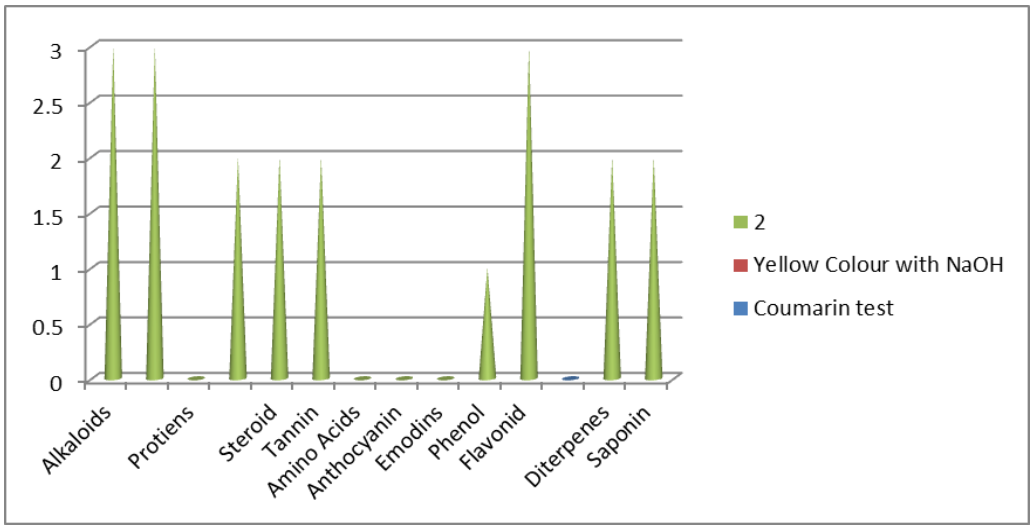


In toluene- 3gm of flower bud add on **Toluene solvent**, heat at 75 to 90 ℃ in soxhlet apparatus, Do phytochemical Analysis.



In toluene-0=absent, 1 = average, 2 = good, 3 = very good

Sr. no		Phytochemical	Observation	Result
		Analysis		
1		Coumarin test	Yellow Colour with NaOH	2
2	Alkaloids	(a)Wagner test	Brown reddish	3
		(b)Hager's test	Yellow color	3
3	Protiens	Cardial Glycosides	Brown Color ring	0
4		Kellerskillumi test		2
5	Steroid	Liberman (5 drops of $CHCl_3$ + 5 drops of H_2SO_4)	Yellow with Green Color	2
6	Tannin	Lead acetate test	Yellow with green	2
7	Amino Acids	Ninhydrin Test	No Change	0
8	Anthocyanin	2N HCl +NH_3	No Change	0
9	Emodins	NH_4OH + Benzene	No Change	0
10	Phenol	Alcoholic $FeCl_3$	Bluish black Color	1
11	Flavonid	NH_4OH test	Yellow Color	3
		Alkaliner test		
12	Diterpenes	Copper Acetate test	Green Color	2
13	Saponin	$FeCl_3$ Solution	Oil Form	2



Fluorescent test for Manikara zapota buds powder

This is a Qualitative analysis test and can be used as a first primary test. When the starting powder material is deal with acid, base and neutral reagents. It may assemble Fluorescent light. In the present qualitative investigation, fluorescent light in observed in Con.HCl,Con.H_2SO_4,con HNO_3 and ammonia as a consequence Organic solvent or when Con. HCl, con H_2SO_4 is used the powder generate fluorescence.

Fluorescent test for Manikara zapota buds powder			
1	Powder + Con-HCl	No Change	0
2	Powder + Con-HNO_3	Light Yellow Color	1
3	Powder + Con H_2SO_4	Dark brown	3
4	Powder + NH_3(l)	Only yellow colour	2
5	Powder + Toluene	No change	0
6	Powder + Benzene	No change	0
7	Powder + Chloroform	Only yellow colour	2

0=absent, 1 = average, 2= good, 3= very good

Identification and Separation of Phytocomponents

Chromatographic Analysis

Column Chromatography

The 15 x 4 cm column equipment was cleaned with water and then rinsed with solvents. A tiny bit of cotton that is fastened to a stand was placed inside the column's lowest section. For one hour, the silica gel (60–120 mesh) was activated in an oven set at 120°C. A 100150g silica gel slurry was made, placed inside the column, and digested using petroleum ether at 60°C80°C. After mixing the ethyl acetate extract with the silica gel slurry mentioned above, the eluents were progressively removed in increasing order of polarity, beginning with toluene, ethyl acetate, and acetone. Ultimately, flavonoids were separated using a 7:3 eluent mixture (MeOH: Water), and the resulting fraction was gathered in test tubes. Bioactive chemicals are frequently purified using column chromatography. In this study, low polar to high polar eluents were used to elute the methanolic floral buds extract of Manilkara zapota. As seen here, the fractions that eluted from toluene, acetone, and ethyl acetate were gathered.

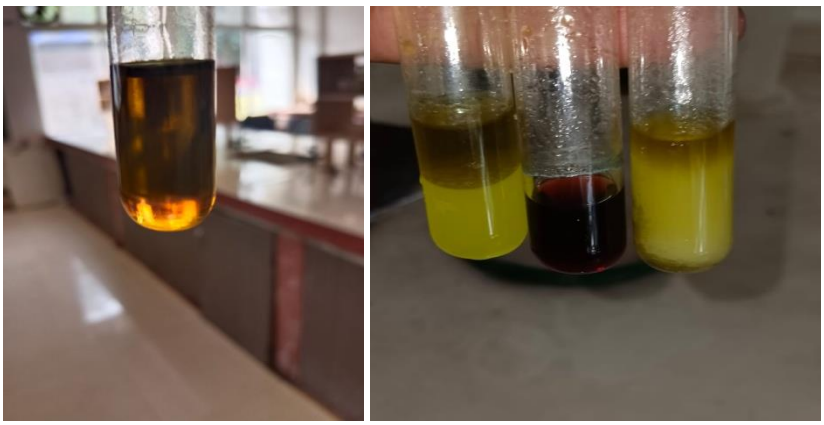


Figure 3a: Collection of fractions (ethyl acetate, acetone and toluene) b) Separation process in column.

Retention Factor (Rf)

The compounds identified from various solvent fractions (Acetone, ethyl acetate, and Toluene) spotted on TLC plate are shown in Table 6. The Rf values obtained were compared with literature data and it is inferred that most of them are flavonoids in nature.[17]

Table 6: Rf value of flavonoids.

S. No	Fractions	Rf	Compound
1.	Acetone	0.62 0.82	Chalcones Kaempferol
2.	Ethyl acetate	0.03 0.67 0.89	Quercetin Apegenin Flavonones
3.	Toluene	0.03 0.68	Unidentified Chalcones

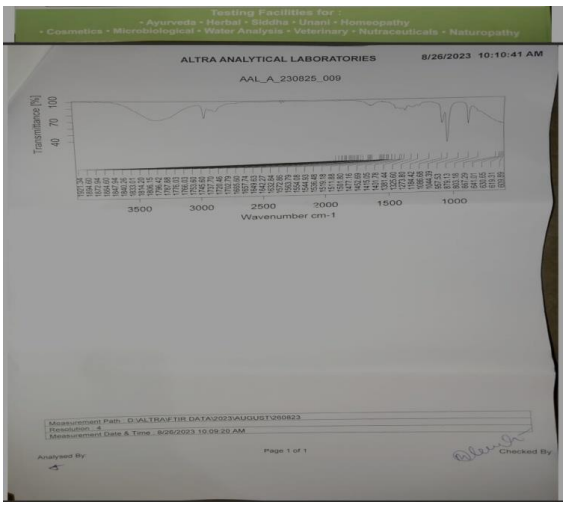
Thin Layer Chromatography

The ethyl acetate fraction is spotted on a TLC plate to find out retention factor (Rf) using the solvent system, n-butanol: Acetic acid: Water (4:1:5). The Acetone gave single spot at (Rf = 0.96), which indicates that it might be due to the presence of the flavonoid. The presence of flavonoid is further confirmed qualitatively from the following analysis.

It gave yellow color on reaction with NaOH, which decolorized on the addition of HCl (Alkaline reagent test) Figure. The compound turned from greenish yellow to deep yellow by spraying ammonia for above fractions. (Figure).

IR –Visible spectroscopy

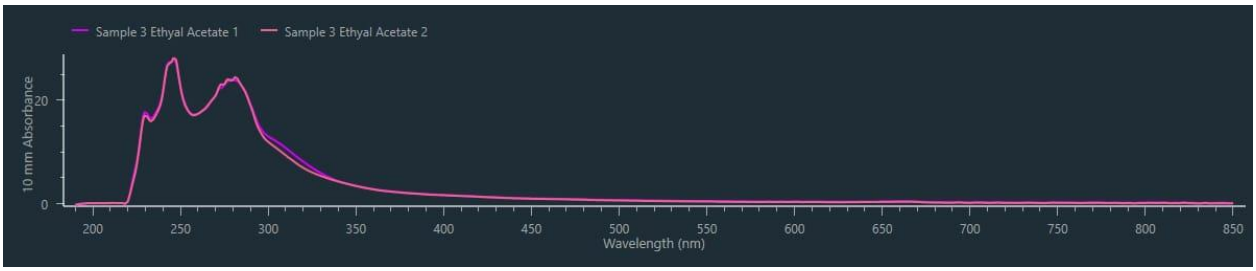
From the occurrence of peaks at 3319 cm^{-1}, 2947-2835 cm^{-1}, 1653 cm^{-1}, 1452 cm^{-1}, 1409 cm^{-1}, 1112 cm^{-1}, 1030 cm^{-1} at 3319 cm^{-1} suggested and further confirmed the presence of the flavonoid as shown in Figure.



FT-IR spectrum isolated flavonoids in flower buds ethyl acetate ftraction of Manikara zatopa

UV- Visible Spectroscopy

The UV – Visible spectra suggested that the absorbance of a compound at λ_{max}= 256 nm, 236 nm and 241 nm is a flavonoid (quercetin) apigenin, flavanone, as shown in Figure.



Quantitative estimation of chemical constituency

2.5.1. Determination of alkaloids

A total of 200 mL of 20% acetic acid was added to 3 g of flower bud powders taken in a separate 250 mL beaker and covered to stand for 5 h. This mixture containing solution was filtered and the volume was reduced to one quarter using water bath. To this sample, concentrated ammonium hydroxide was added drop-wise until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed. The percentage of total alkaloid content was calculated as.[14-15]

Percentage of total alkaloids in Flower bud (%)=Weight of residue×100/Weight of sample taken

=1.037×100/1.112

=93.25%

The quantitative determination of the chemical constituency

The percentage of yield of crude successive extract in Acetone and Ethyl Acetate, Toluene, flower bud parts of Manilkara zapota. In Acetone extract of flower bud is higher yield (22.6%). Followed by ethyl acetate extract (15.4%). The toluene extract show the lowest yield of (6.3%).

Percentage yield, total flavonoid content of polar and non polar solvent extract of flower bud of Manilkara zapota

Sample	***Total Flavonoids***
Acetone	22.6%
Ethyl Acetate	15.4%
Toluene	6.3%

CONCLUSION

Phytochemical analysis of Manilkara Zapota tree flower bud was done in polar and non-polar solvents using Acetone, Ethyl acetate as polar solvent, Toluene as non-polar solvent. Studying Phyto Chemical Analysis revealed that Alkaloids, Glucoside, Flavonoids, Coumarin, Phenol, Tannin Emodins, Anthocyanin were present while compounds like Amino acid were absent. The scientist as well as Medicines, Ayurveda are best alternative to combating for diseases they have immense potential to treat the plant the least sides effects and with highly safety and efficacy in Manilkara Zapota Flower bud. Their presence is further confirmed by histochemical and fluorescence studies. Using

chromatographic techniques such column chromatography and thin layer chromatography, the isolated molecule was separated and identified. The methanol: water fraction's active flavanoids were easily separated using the subsequent eluting process. Determine % of flavonoids and alkaloids by quantitative analysis.

The percentage of crude successive extract yield in Manilkara zapota flower bud parts, acetone, ethyl acetate, and toluene. The yield is higher (22.6%) in flower bud acetone extract.Ethyl acetate extract (15.4%) comes next. The lowest yield, 6.3%, is shown by the toluene extract.Flower Buds of this plant are also used for the purpose of quality control of herbal medicines.

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