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QUALITATIVE ESTIMATION OF PHYTOCHEMICALS IN FIVE LEAF EXTRACTS

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ABSTRACT:

Phytochemistry is the study of phytochemicals. Ancient history points out the use of various plant parts for the treatment of diseases in humans and animals the science of which is known as ethnopharmacology. In the present study, five different types of leaves like guava, vitex, lemon, custard apple and moringa were estimated for qualitative phytochemical analysis. Methanol, ethanol and aqueous extracts were prepared from five different leaves and subjected for qualitative phytochemical screening. The phytochemical tests analysed were alkaloids, glycosides, tannins, phenols, flavonoids, saponins, terpenoids, gums and resins and phytoserols. The alkaloid results was observed as negative in aqueous extract of all leaves except custard apple leaves. The methanol and ethanol extracts of guava, vitex and moringa leaves showed positive results. The glycosides were present in all three extracts of guava leaves, aqueous extract of moringa, lemon and custard apple leaves. The tannins were present in methanolic extract of guava and aqueous extract of moringa leaves. The phenols content was negative in ethanol extract of custard apple leaves and in methanol, ethanol of moringa leaves extract. Methanol extracts of guava, vitex, lemon, custard apple leaves and aqueous extracts of lemon, guava, custard apple and moringa leaves showed positive results for flavonoids. Saponins were present in all three extracts of guava and lemon, methanolic and aqueous extracts of vitex, ethanolic and aqueous extracts of moringa and absent in all the extracts of custard apple leaves. The ethanolic extract of guava leaves, ethanolic and aqueous extract of vitex leaves, methanolic extract of custard apple leaves, methanolic and ethanolic extracts of moringa leaves showed negative results for terpenoids. The phytosterols presence was observed in methanol and ethanol extract of guava leaves, vitex leaves and ethanolic extract of lemon leaves. The presence of gums and resins was observed in aqueous extracts of all leaves and absent in methanol and ethanol extracts of all leaves except the methanolic extract of lemon leaves.

Keywords: Phytochemicals, qualitative analysis, alkaloids, glycosides, tannins, phenols, flavonoids, saponins, terpenoids, gums and resins

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INTRODUCTION

Plants are a rich source of active biological compounds called phytochemicals, which have been used by many industries such as the pharmacological industry, cosmetic industry, food and beverage industry, ayurvedic and herbal medicines, etc., for various purposes. Albrecht Kossel, Nobel Prize winner for physiology or medicine in 1910, was the first one to come up with the notion of secondary metabolites (Hussein and El-Anssary, 2019). A lot of medical properties such as antifungal, antibacterial, and antiviral properties are attributed to them. The secondary metabolites from plants are safe for human consumption, hence a lot of researchers are trying to search for nature-friendly alternatives that can boost human health, in the form of herbal medicines, natural colorants in the food, cosmetic, and skincare sectors, natural colours for dyeing clothes in the fashion industry. The research has now expanded into the use of plant extracts for the synthesis of nanoparticles.

REVIEW OF LITERATURE

Plants are enriched with different phytochemicals like vitamins, phenolic acids, terpenoids, lignins, tannins, flavonoids, stilbenes, quinones, alkaloids, coumarins, amines, betalains, and other metabolites are all abundant sources of free radical scavengers in plants. They are also anti-inflammatory, anticancer, anti-atherosclerotic, anticarcinogenic, antimutagenic, antibacterial and antiviral antioxidant substances (Ajiboye *et al*., 2013). Plants produce a wide range of natural products or secondary metabolites that play an important role in protecting against predators and microbial pathogens due to their toxic nature and repellence to herbivores and microbes, and some of which are also involved in defence against abiotic stress (e.g. UV-B exposure) (Schafer and Wink, 2009). Plants are capable of producing a wide range of bioactive chemicals. Fruits and vegetables acquire high levels of phytochemicals, which may protect against free radical damage (Suffredini *et al*., 2004).

Plants are natural sources of antioxidants, among them carotenoids, tocopherols, flavonoids, vitamin C and other phenol compounds are best known. Recently flavonoids received the highest attention among the natural antioxidant. Dietary flavonoids are considered to be more effective antioxidants than vitamins C and E (Dehshahri *et al*., 2012) Guava leaves extensively used for their anti-inflammatory, cough sedative, antidiarrheic, antispasmodic, antiobesity, antihypertension, and antidiabetic properties. Guava leaves bioactive and therapeutic characteristics are principally determined by the presence of a distinct diversity of bioactive polyphenolic chemicals, such as quercetin and other flavonoids, as well as ferulic, caffeic, and gallic acids. Secondary metabolites are phenolic substances that have high antioxidant and immunostimulant properties (Kumar *et al.*, 2021a).

Moringa oleifera leaves due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics, and carotenoids, showed antioxidant activity that was stable at pH 4 and 9. As a result, this plant extract is a potential source of natural dietary antioxidants in food supplements. Moringa leaf juice can lower blood sugar levels and has purgative, anti-inflammatory, and antimalarial effects. It can also help with

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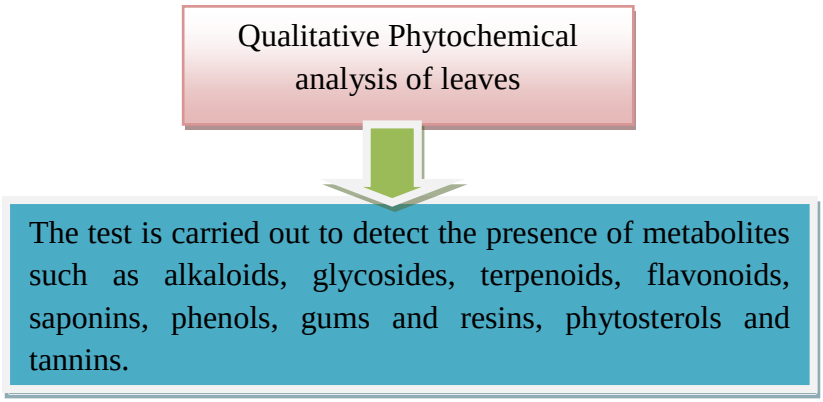
piles, sore throats, fevers, catarrh, bronchitis, eye and ear infections, as well as wound healing and relieve of headache (Aliyu *et al*., 2016). The beneficial health-promoting potentials of vitex agnus-castus, including antioxidant, immunomodulatory, cytotoxic, antimutagenic, antimicrobial, antifungal, antinociceptive, opioidergic, antiepileptic, and anti-inflammatory properties. In vitro activities of vitex agnus-castus leaves of methanol extract(400 mg/kg) shows the Anti-inflammatory effect which reduced IL-6 and TNF-α levels (Souto *et al*., 2020)

Citrus leaves are rich source of bioactive compounds including antioxidants such as flavonoid, ascorbic acid, and phenolic compounds and recently recommended the use of antioxidant vegetable extracts both as an alternative to food preservation technology and as prophylactic agents for some human diseases (Khettal *et al*., 2017). *Annona squamosa* leaves (ASLs) due to their extensive pharmacological properties and biological activities, such as antioxidant, antidiabetic, antiviral, antimicrobial, anticancer and hepatoprotective activities. These activities are due to the glycosides, phytosterols, carbohydrates, oils, saponins, tannins, alkaloids, phenols, flavonoids, peptides, and various acetogenin compounds (Kumar *et al*., 2021b). In the present study, the extraction and identification of phytochemicals in five different leaves sources (guava, vitex, lemon, custard apple, moringa) were estimated in different solvents like methanol, aqueous and ethanol.



MATERIALS AND METHODS:

1. **Collection of plant material:** Fresh leaves of guava, vitex, lemon, custard and moringa were collected from the area of Osmania University, Hyderabad, Telangana. The leaves were washed with running tap water, shade dried and pulverised to fine powder in mixer. The grinded leaves powder was sieved and stored in air tight container for ethanol, aqueous and methanolic extraction.
2. **Chemicals required:** Mercuric chloride, Potassium iodide, iodine, picric acid, ammonia, sulphuric acid, chloroform, glacial acetic acid, ferric chloride, lead acetate, ferric chloride, sodium nitrite, sodium hydroxide, quercetin standard, aluminium chloride, gallic acid standard, Folin-Ciocalteu, sodium carbonate, Folin-Denis reagent, tannic acid standard, petroleum ether, methanol, ethanol.
3. **Preparation of sample extract:** Allethanol, methanol and aqueous extractions were done in the ratio 1:15 i.e., 1 part of leaf powder and 15 parts of solvent. The methanolic extract of leaves was done by Soxhlet method. The five dried leaf powders (27 gm each) were packed in a muslin cloth each for placing in the Soxhlet thimble,

which is placed inside the Soxhlet extractor. Methanol (400 mL) was taken in the round bottom flask of the Soxhlet apparatus. The solvent is heated through isomantle. Upon heating, methanol evaporates, moves through the apparatus to the condenser. The condensate drips into the extractor. When the solvent level reaches the siphon, it then falls into the flask. This is one cycle of extraction. Extraction was done for 8 hours. The feed to solvent ratio was kept at 1:15 and was same for all the five leaf powders used in the study. The extract obtained was in dark greenish colour and was stored in a glass bottle in a refrigerator. This extract was utilised for subsequent analysis.

For ethanol extraction the leaf powders were steeped in ethanol for 48 hours at room temperature and covered properly to prevent evaporation. The mixture was stirred thoroughly and filtered through whatmann filter paper and stored for further analysis (Kemabonta *et al*., 2014)

The aqueous extraction was prepared with modification of procedure done by (Alapati and Sulthana, 2015). The leaf powders were placed in conical flasks with distilled water 1:15 feed to solvent ratio and boiled for 15-20 minutes. The flasks were then removed, allowed to cool and then filtered using whatmann filter paper. The filtrate was stored and used for further analysis.

4. Qualitative estimation of phytochemicals: The qualiatative phytochemical estimation was done by the procedures as per (Harbourne, 1993).

4.1 Test for Flavonoids: 5 ml ammonia was added to each portion of the sample extract, followed by a few drops of concentrated sulphuric acid. The appearance of a yellow coloration indicates the presence of flavonoids, and it disappeared after standing for a while.

4.2 Test for Terpenoids: 2 ml chloroform and 3 ml concentrated sulphuric acid were added to 5 ml of each extract to form a reddish brown monolayer at the interface. This confirmed the presence of terpenoids.

4.3 Test for Saponins: Each extract was mixed with 20 ml distilled water and agitated for 15 minutes in a graduated cylinder. Saponins presence was were confirmed by the formation of a 1cm layer of foam.

4.4 Test for Tannins: A few drops of 1% Lead acetate were added to 5 ml of extract, and the formation of a yellow precipitate confirmed the presence of tannins.

4.5 Test for Phenols:

a)Ferric chloride test: For the presence of phenols, a fraction of each of the extracts was treated with a few drops of 5% Ferric chloride and observed for the formation of a deep blue or black colour.

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b) Liebermann's test: The extract was heated with a sodium nitrite and concentrated H2SO4 solution, then diluted in water, cooled, and added with an excess of dilute NaOH. The presence of phenols was indicated by the formation of a deep red or green, or blue colour.

4.6 Test for alkaloids:

a) **Mayer's test:**1% HCL and 6 drops of Mayer's reagent (1.366g mercuric chloride and 5 g potassium iodide in 100 ml water) were added to a fraction of the extract. The presence of an organic precipitate in a sample indicates the presence of alkaloids.

b) **Wagner's test:** Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml water) was treated with a fraction of the extract, and the development of cream-colored precipitate was observed.

c) **Hager's test:** A few ml of extract was treated with Hager's reagent (saturated aqueous picric acid solutions) and formation of prominent yellow coloured precipitate was observed.

4.7 Test for glycosides: To a small quantity of plant extract (5 ml) 2 mL of glacial acetic acid was added. Later, one drop of ferric chloride solution was added. One mL of concentrated H_2SO_4. was added to the test tube. The deoxy sugar characteristic of cardenolides was shown by the formation of brown ring at the interface. In the acetic acid layer, a violet ring may appear below the brown ring, while a greenish ring may form gradually across the thin layer.

4.8 Test for gums and resins: Under continual stirring, around 10ml of the extract was added slowly to 25ml of pure alcohol. Gums and resins presence indicates the formation of precipitation(Harborne and Baster, 1993)

4.9 Test for phytosterols: (Shaikh and Patil, 2020)

a) **Salkowski's test:** Equal quantity of chloroform is treated with plant extract(filtrate) with few drops of concentrated H_2SO_4 (shaken well and allowed to stand). Red colour in the lower layer indicates the presence of phytosterols.
b) **Hesse's response:** 5 ml aqueous extract was added with 2ml of chloroform and 2 ml conc. H_2SO_4, formation of pink ring/red colour (in lower chloroform layer) indicates the presence of phytosterols.

RESULTS AND DISCUSSION:

The phytochemical screening of five leaf sources of guava, vitex, lemon, custard apple and moringa are given Table 1. The alkaloids results was observed as negative in aqueous extract of all five leaves except custard apple leaf. The methanol and ethanol extracts of guava (Mayer's test, Wagner's test, Hagers's test), methanol (Mayer's test, Wagner's test)

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and ethanol (Mayer's test) extract of vitex and methanol (Mayer's test) and ethanol (Mayer's test, Hagers's test) extract of moringa leaves showed positive results.

The results showed the absence of alkaloids in all the extracts of lemon leaves. The glycosides were present in all three extracts of guava leaves, aqueous extract of moringa, lemon, custard apple leaves and absent in all the extracts of vitex leaves. The tannins were present in methanolic extract of guava and aqueous extract of moringa leaves. In ferric chloride test, the results indicate the phenol content as negative in ethanol and aqueous extracts of lemon leaves, methanol and ethanol extracts of custard apple leaves and in moringa leaves. In Liebermann's test, the phenol content was observed as negative in ethanol and aqueous extract of vitex leaves, ethanol extract of custard apple leaves and absence in all the extracts of moringa leaves.

Methanol extracts of guava, vitex, lemon, custard apple leaves and aqueous extracts of lemon, guava, custard apple and moringa leaves showed positive results for flavonoids. Saponins were present in all three extracts of guava and lemon leaves, methanolic and aqueous extracts of vitex leaves, ethanolic and aqueous extracts of moringa and absent in all the extracts of custard apple leaves. The results showed the presence of terpenoids in methanol leaves extract of guava, vitex, lemon and ethanol leaves extract of lemon, custard apple and aqueous extract of all leaves expect vitex. The terpenoids presence was shown in all three extracts of lemon leaves.

In Salkowski's test, the methanol leaves extract of guava, vitex and ethanol extract of guava and lemon showed positive results for phytosterols and in Hesse's response the methanol and ethanol extract of guava and ethanol extract vitex showed the presence of phytosterols. The presence of gums and resins was observed in aqueous extracts of all leaves and absent in methanol and ethanol extracts of all leaves except the methanol extract of lemon leaves.

The results indicates the presence of all phytochemicals tested in guava leaves in methanolic extract were similar with the results obtained by (Kenneth *et al*., 2017) and ethanol extracts of saponins, glycosides were opposite with results obtained and similar results were seen in aqueous extract of glycosides, flavonoids, saponins and terpenoids.

The similar results of alkaloids, tannins and opposite results of glycosides, flavonoids were obtained in aqueous extract of vitex leaves and opposite in methanolic extract of vitex leaves in alkaloids, flavonoids, glycosides, tannins and similar in presence of phenolic compounds (Pawar and Kamble, 2017). In ethanol extract of lemon leaves, phenols (Liebermann's test) and saponins results obtained were similar and opposite were found in alkaloids, flavonoids, tannins and glycosides. In aqueous extract, the similar results were obtained with phenols (Liebermann's test), flavonoids, saponins and resins (Ewansiha *et al*., 2016).

The custard apple leaf ethanol extract results were opposite with results obtained by (Nguyen *et al*., 2020) and the similar results of aqueous extract was observed in alkaloids, glycosides, phenols, flavonoids (Nguyen *et al*., 2020). The ethanolic extract of moringa

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leaves were similar with the results of alkaloids, saponins (Sudha *et al*., 2020) and the results obtained for methanol extract were opposite (Unuigbe *et al*., 2015).

Table 1 Qualitative phytochemical analysis of different leaves extracts

Tests	Guava *Psidium guajava)*			Vitex *(Vitex agnus-castus)*			Lemon *(Citrus limon)*			Custard Apple *(Annona reticulate)*			Moringa *(Moringa oleifera)*		
	M	**E**	**A**	**M**	**E**	**A**	**M**	**E**	**A**	**M**	**E**	**A**	**M**	**E**	**A**
1.Alkaloids															
a)Mayer's test	+	+	-	+	+	-	-	-	-	-	-	++	+	+	-
b)Wagner's test	++	+	-	+	-	-	-	-	-	-	-	-	-	-	-
c)Hagers's test	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-
2.Glycosides	+	+	+	-	-	-	-	-	+	-	-	+	-	-	+
3.Tannins	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+
4.Phenols															
a)Ferric chloride test	++	++	++	+	+	+	+	-	-	-	-	+	-	-	++
b)Liebermann's Test	++	++	++	+	-	-	+	+	+	+	-	++	-	-	-
5.Flavonoids	+	-	+	+	-	-	+	-	+	+	-	+	-	-	+
6.Saponins	++	++	++	+	-	++	+	+	++	-	-	-	-	+	++
7.Terpenoids	++	-	+	+	-	-	+	+	+	-	+	+	-	-	+
8. Phytosterols															
a)Salkowski's test	+	+	-	+	-	-	-	+	-	-	-	-	-	-	-
b)Hesse's response	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-
8.Gums and resins	-	-	+	-	-	+	+	-	++	-	-	++	-	-	++

++ = Highly present; + = present; - = absent M= Methanolic extract, E= Ethanolic extract, A= aqueous extract.

CONCLUSION

Phytochemicals are low molecular weight substances, that possess a multitude of health benefits. Currently, an estimate of 100,000 substances is known to mankind, the consumption of which is touted to be having various health benefits, which are being recognized worldwide. The present study qualitative was helpful in determining the presence of phytochemicals in the three types of leaf extracts. Qualitative estimation of alkaloids, glycosides, tannins, phenols, flavonoids, saponins, terpenoids, gums and resins and phytoserols in aqueous, methanol and ethanol leaf extracts were estimated. Guava methanolic and ethanolic leaf extracts showed the presence of all the phytochemicals under study, while lemon and custard apple methanolic and ethanolic leaf extracts showed a negligible amount of alkaloids, glycosides and tannins. This could be attributed to the type of extraction

employed, the season in which the leaves were collected, the plant maturity stage, the geographical location of the plant.

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