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Qualitative Phytochemical Screening Methods of Bioactive Compounds from Medicinal Plants

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Abstract: Medicinal plants have a great significance as a potential source of bioactive secondary metabolites including alkaloids, flavonoids, terpenoids, phenolic compounds, tannins, and saponins which have the capabilities to defend the plant and humans from diseases. These valuable bioactive compounds have greatly contributed to the advancements in the discovery of new drugs. For example, antimicrobial resistance has been a major problem in the healthcare industries, and bioactive compounds found in plants have the potential to combat this antimicrobial resistance. These compounds have new chemical structures and multiple medicinal applications. Many diseases have the likelihood to be fought through the synergies obtained from secondary metabolites in plants. This review presents the various qualitative techniques applied in screening the phytochemical compounds from medicinal plants. The review discussed about the standard chemical tests that are widely used in identifying major classes of secondary metabolites, such as alkaloids, flavonoids (Shinoda and alkaline reagent tests), tannins and phenolics (ferric chloride and lead acetate tests), saponins (frothing test), glycosides (Keller-Killiani and Borntrager tests), steroids and terpenoids (Liebermann-Burchard and Salkowski tests). These techniques are important due to their simplicity, speed, and low cost and therefore can be used in preliminary phytochemical analyses, particularly in resource-constrained environments.

1. Introduction

Qualitative photochemical screening methods are an important aspect of studying medicinal plants, as they provide preliminary information about the chemicals present in the plant extracts (Suresh *et al.*, 2025). These methods allow the identification of major classes of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, phenolics, and glycosides, many of which are responsible for therapeutic effects in humans (Katsriku *et al.,* 2025). These methods are rapid, cost-effective, and easy to perform; thus, they can be rendered quite suitable for preliminary phytochemicals screening, particularly in resource-limited laboratories. The results help in the validation of the ethnomedicinal applications and biological activities like antioxidant, antimicrobial, anti-inflammatory, or anticancer effects of extracts (Nortjie *et al.,* 2022; Bouammali *et al.,* 2023; Haddou *et al.,* 2023). The qualitative screening methods also guidefor further research, including

quantitative phytochemicals screening, isolation, purification, and structural characterization of active compounds. Additionally, it supports quality control and standardization of herbal drugs by confirming the presence of characteristic constituents (Abilkassymova and Aldana-Mejía, 2024).

The application of plant extracts is growing actively in the world because of the existence of biologically active compounds that assist in food preservation and the inhibition of many diseases because of their antimicrobial and antioxidant capabilities (ziziaram *et al.,* 2021; Ezeonu *et al*., 2016; Danjuma *et al.,* 2025). The use of natural products in traditional practices to treat diseases has led to the realization of the need to search on new therapeutic drugs1 of these plants. The medical value of plants is determined by the kind of phytochemical constituents, which they contain exhibiting different physiological activities on the human body (Doley *et al.,* 2016; Louafi *et al.,* 2025; Ouahabi *et al.,* 2025). The phytochemical screening technique, therefore, could be utilized to identify these compounds that are found in plants that can be utilized as the foundation of the modern drug development. Phytochemical screening, a rapid, low-cost chemical assay of bioactive compounds (phytochemicals) in plants, identifies classes such as alkaloids, flavonoids, tannins and saponins, to learn about medicinal properties, drug discovery by revealing active constituents, which can be further tested against a disease or microbe, and by simple color tests or elaborate chromatography (Alavi *et al*., 2022; El Hassania *et al.,* 2024). As a result of the growth in the number of multi-drug resistant bacteria, the availability of anti-biotics and their affordability has been made challenging across the globe (Danjuma and Lawan, 2025). Therefore, the efficacy of the treatment is minimized leading to increment of the mortality rate, disease incidence and health care expenditure (Kabir and Lawan, 2025). It is observed that the ratio of the resistance of microbes to synthetic drugs is increasing, and the number of newer antimicrobial drugs is decreasing (Al-Hamoud *et al*., 2022; Danjuma, 2025).

In dealing with these microbial infections, the emphasis has been shifted to the achievement of new, effective and low-cost drugs to deal with microbial infections that are high particularly in developing countries where 50% of the death rate is caused by infectious diseases (Sharma *et al.,* 2020; Kabir*et al.,* 2025). Phytochemicals are the substances that were produced because of the metabolic processes that took place in plants (Elisha *et al.,* 2017). Thus, the necessity to find innovative antibiotics in natural resources is extremely emphasized in the sphere of modern medicine that could assist in the process of overcoming the unfavorable socio-economic and health outcome brought about by multi-drug resistant microbes (Mostafa *et al.,* 2018). This review provided the various ways of phytochemical screening of medicinal plants.

2. Methods of Phytochemical Screening of Extracts

2.1 Detection of alkaloids

2.1.1 Dragendroff's/ Kraut's Test

Alkaloids can be detected using Dragendroff's test in which a few mL of the sample filtrateis added to 1-2 mL of *Dragendorff's reagent.* An appearance of a reddish-brown precipitate confirms the presence of alkaloids (Silva *et al.,* 2017; Singh and Kumar, 2017).

2.1.2 Hager's Test

To qualitatively check the presence or absence of alkaloids using Hager's test, few mL of the sample filtrateis added to1-2 mL Hager's reagents. An appearance of a white precipitate indicates the presence of alkaloids (Junaid and Patil, 2020).

2.1.3 Mayer's/ Bertrand's/ Valser's test

Mayer's test involves adding few mills of the sample filtrate in a test tube followed by addition of1-2 drops of *Mayer's reagent*. An appearance of a creamy white/yellow precipitate indicates that alkaloid is present. This test is also called Bertrand or Valser's test (Juanid and Patil, 2020).

2.1.4 Wagner's Test

Wagner's test of alkaloids involves addition few mL of the sample filtrate followed by addition of 1-2 drops of *Wagner's reagent* in a test tube. Formation of brown/reddish precipitate shows the presence of alkaloids (Singh and Kumar, 2017).

2.2 Detection of Proteins/Amino Acids

2.2.1 Biuret test

To detect proteins using Biuret test, 2 mL filtrate is added to 1 drop of 2 % copper sulphate solution, followed by addition of 1 mL of 95% ethanol and then few potassium hydroxide (KOH) pellets. A formation of a pink color solution in ethanolic layer indicates the presence of proteins (Silva *et al.,* 2017, Junaid and Patil, 2020).

2.2.2 Millon's test

In Million's test, 2 mL of the sample filtrate is mixed with few drops of *Millon's reagent.* An appearance of a white precipitate shows the presence of proteins (Junaid and Patil, 2020).

2.2.3 Ninhydrin test

In Ninhydrin test, 2 mL of the sample filtrate is mixed with 2 drops of Ninhydrin solution (which is a combination of 10 mg ninhydrin and 200mL acetone). A purple color formation solution shows Amino acids (Junaid and Patil, 2020).

2.2.4 Xanthoproteic test

The sample plant extract is added to a few drops of concentrated nitric acid solution. A formation of a yellow coloured solution shows the presence of proteins (Silva *et al*., 2017, Junaid and Patil, 2020).

2.3 Detection 0f Flavonoids

2.3.1 Alkaline reagent test

In alkaline test for flavonoids, 1 mL of the sample extract should be mixed with 2mL of 2% sodium hydroxide (NaOH) solution and then few drops dilute hydrochloric acid (HCl) are added. Formation of an intense yellow colour solution which becomes colourless on addition of diluted acid shows the presence of flavonoids (Gul *et al*., 2017).

2.3.2 Lead acetate test

In lead acetate test, a sample plant extract is added to 10% ammonium hydroxide solution. A formation of yellow fluorescence indicates the presence of flavonoids (Junaid and Patil, 2020).

2.3.4 Shinoda's test/ Mg-hydrochloride reduction test

In this method, 1 mL plant extract is added to a few drops of 10 % lead acetate solution. An appearance of a yellow precipitate shows the presence of flavonoids (Junaid and Patil, 2020).

The plant extract is dissolved in 5mL alcohol followed by addition of fragments of magnesium ribbon and few drops of concentrated hydrochloric acid (HCl). Formation of a pink to crimson colored solution shows the presence of flavonal glycosides (Junaid and Patil, 2020)

2.3.3 Shibata's reaction/ Cyanidin test

One milligram (1gm) of the aqueous solution of the extract is dissolved in 1-2 mL 50 % methanol by heating followed by addition of metal magnesium and 5-6 drops of concentrated hydrochloric acid (HCl). Formation of red color reveals the presence of flavonols, while orange colour shows flavones (Kumar *et al*., 2018)

2.3.4 Ferric chloride test

An aqueous solution of the extract is mixed with few drops 10 % ferric chloride solution. An appearance of green precipitate confirms the presence of flavonoids (Junaid and Patil., 2020).

2.3.5 Pew's test

Few mL aqueous extract solution is added to 0.1gm metallic zinc followed by 8 mL concentrated sulphuric acid. A red colour formation shows the presence of flavonols (Pant et al., 2017).

2.3.6 Zinc-hydrochloride reduction test

In this test, the plant extract is mixed with pinch of zinc dust followed by addition of concentrated HCl along the side of test tube. Formation of Magenta colour shows the presence of flavonoids (Kumar *et al.,* 2018).

2.3.7 Ammonia test

The sample filtrate is added to 5 mL dilute ammonia solution followed by addition of concentrated sulphuric acid. A yellow colour formation shows the presence of flavonoids (Tyagi*et al.,* 2017).

2.3.8 Concentrated Sulphuric Acid Test

The sample plant extract is mixed with few drops of concentrated sulphuric acid. An appearance of orange colour shows the presence of flavonoids (Pant *et al.,* 2017).

2.4 Test of Phenolic Compounds

2.4.1 Iodine test

In this test, 1 mL of the sample extract is added to a few drops of dilute Iodine solution. Formation of a transient red colour indicates the presence of phenolic compounds (Singh *et a*l., 2017).

2.4.2 Ferric chloride test

In this test, an aqueous solution of the extract is mixed with few drops 5 % ferric chloride solution. Formation of a dark green/bluish black colour indicates the presence of phenolic compounds (Junaid and Patil, 2020)

2.4.3 Gelatin test

The sample plant extract is dissolved in 5 mL distilled water followed by addition of 1 % gelatin solution and 10 % sodium chloride solution, NaCl. A white precipitate formation shows the presence of phenolic compounds (Junaid and Patil, 2020; Aourabi *et al.,* 2021).

2.4.4 Lead acetate test

In this test, the sample plant extract is dissolved in 5 mL distilled water followed by addition of 3 mL of 10 % lead acetate solution. Formation of a white precipitate shows the presence of phenolic compounds (Junaid and Patil, 2020)

2.4.5 Ellagic Acid Test

In this test, the aqueous solution of the sample plant extract is added to 5 % glacial acetic acid followed by addition of 5 % sodium nitrite solution. If the Solution turns muddy / Niger brown precipitate, phenolic compounds are present (Junaid and Patil, 2020).

2.4.6 Potassium dichromate test

In this method, the sample plant extract is usually added to a few drops of potassium dichromate solution. A dark coloration confirms the presence of phenolic compounds (Kumar *et al*., 2018).

2.4.7 Hot water test

Warm water in beaker is added to the mature plant part followed by warming for a minute. Formation of a black or brown colour ring at the junction of dipping indicates the presence of phenolic compounds (Pooja and Vidyasagar, 2016).

2.5 Test for Cartenoids

In this test, 1 gm of the sample extract is mixed with 10 mLchloroform which is then vigorously shaken and filtered. The filtrate is added to a few drops of concentrated sulphuric acid. Formation of a blue colour at the interface indicates the presence of carotenoids (Tyagi, 2017)

2.6 Detection of Tannins

2.6.1 Gelatin test

In this test, the sample of the plant extract is dissolved in 5 mL distilled water followed by addition of 1 % gelatin solution and 10 % sodium chloride, solution NaCl. Formation of a white precipitate indicates the presence of tannins (Junaid and Patil, 2020)

2.6.2 Braymer's Test

In this test, 1 mL of the sample filtrate is mixed with 3mL distilled water followed by addition of a 3 drops 10 % Ferric chloride solution. Formation of a blue-green colour shows the presence of tannins (Uma *et al*., 2017)

2.6.3 10% NaOH Test

In this test, a quantity 0.4mL of the plant extract is added to 4 mL of 10 % sodium hydroxide solution, NaOH followed by shaking the mixture vigorously. Formation of emulsion indicates the presence of hydrolysable tannins (Uma *et al.,* 2017).

2.6.4 Bromine water test

In this method, a quantity of 10 ml of bromine water is added to 0.5gm plant extract. Decoloration of bromine shows the presence of tannins (Junaid and Patil, 2020).

2.6.5 Lead sub acetate test

In this test, 1 mL filtrate is mixed with 3 drops of lead sub acetate solution. A creamy gelatinous precipitate confirms the presence of tannins (Uma *et al.,* 2017).

2.6.7 Mitchell's Test

In this test, the sample extract solution is added with iron, followed by addition of sodium tartarate and ammonium acetate solution. A water-soluble iron-tannin complex, which is insoluble in solution of ammonium acetate shows the presence of tannins (Uma *et al.,* 2017)

2.7 Carbohydrates

2.7.1 Barfoed's Test

In this case, 1 mL of the sample filtrate is added to 1 mL Barfoed's reagent followed by heating for 2 minutes. Formation of red precipitate shows the presence of monosaccharides **(**Junaid and Patil, 2020**)**

2.7.2 Molish's Test

In this test, 2 mL of the filtrate is added to 2 drops of alcoholic α–naphthol followed by addition of 1 mL concentrated sulphuric acid solution along the sides of test tube. Formation of a violent ring shows the presence of carbohydrates (Junaid and Patil, 2020)

2.7.3 Seliwanoff's Test

In this test, 1 mL of the sample extract solution is added to a 3 mL seliwanoff's reagent followed by heating the mixture on water bath for 1 minute formation of a rose red colour shows the presence of ketoses (Junaid and Patil, 2020)

2.7.4 Resorcinol Test

In this test, 2 mL of the aqueous extract solution is added to a few crystals of resorcinol followed by equal volume of concentrated hydrochloric acid and heated. Formation of a rose colour indicates the presence of ketones (Junaid and Patil, 2020)

2.7.5 Test for Pentoses

In this test, 2 mL concentrated HCl is added to a little amount of phloroglucinol followed by addition of an equal amount of aqueous extract solution and then heated over flame. Formation of a red color shows the presence of pentose (Junaid and Patil, 2020)

2.7.6 Test for starch

In this test, an aqueous extract of the sample is added to a 5 mL 5 % KOH solution. Formation of a cinary colouration shows the presence of starch (Junaid and Patil, 2020)

2.8 Detection of Reducing Sugar

2.8.1 Benedict's Test

In this method, a quantity 0.5mL of the filtrate is added to a 0.5 mL Benedict's reagent followed by boiling for 2 minutes. Formation of green/yellow/red colour shows the presence of reducing sugar (Junaid and Patil, 2020)

2.8.2 Fehling's Test

In this test, 1 mL each of Fehling's solution A and B is added to a 1 mL of the sample filtrate followed by boiling in water bath. Formation of a red precipitate indicates the presence of reducing sugar (Junaid and Patil, 2020)

2.9 Detection of Glycosides

2.9.1 Borntrager's Test

In this test, 2 mL of the filtrated hydrolysatec is added to a 3 mL of Chloroform and shaken vigorously.

The Chloroform layer is separated and 10% Ammonia solution is added. Formation of a pink coloured solution indicates the presence of glycosides (Junaid and Patil, 2020)

2.9.2 Modified Borntrager's Test

The sample plant extract is added to a ferric chloride solution followed by boiling for 5minutes and then cooled and an addition of an equal volume of benzene; the benzene layer is separated and Ammonia solution is added. Formation of a rose-pink to blood red coloured solution indicates the presence of glycosides (Junaid and Patil, 2020)

2.9.3 Legal's Test

This is done by dissolving 50gm of the plant extract in pyridine followed by addition of Sodium nitroprusside and 10% Sodium hydroxide. A pink coloured solution formation shows the presence of glycosides (Junaid and Patil, 2020)

2.9.4 10% Sodium Hydroxide (NaOH) Test

This involves addition of 1 mL of dilute sulphuric acid solution and 0.2mL of the extract followed by boiling for 15minutes. The mixture is allowed cooled and then neutralized with 10% NaOH and 0.2mL Fehling's solution A and B. Formation of a brick red precipitate shows the presence of glycosides (Junaid and Patil, 2020).

2.9.5 Aqueous Sodium Hydroxide Test.

The alcoholic sample extract is dissolved in 1mL of water followed by addition of a few drops of aqueous NaOH solution. A yellow colour formation shows the presence of glycosides (Junaid and Patil, 2020)

2.9.6 Concentrated Sulphuric Acid Test

A quantity of 5 ml of the plant extract is added with 2 mL glacial acetic acid followed by addition of a drop of 5 % FeCL3 and concentrated sulphuric acid. A brown ring formation indicates the presence of glycosides (Junaid and Patil, 2020)

2.9.7 Raymond's Test

In this test, an extract solution is mixed with dinitrobenzene in hot methanolic alkali. Formation of a violet colour indicates the presence of glycosides (Junaid and Patil, 2020).

2.10 Detection of Cardiac Glycosides

2.10.1 Keller-Killani test

In this test, 1 mL filtrate is mixed with 1.5mL glacial acetic acid followed by addition of 1 drop of 5% ferric chloride and concentrated sulphuric acid along the side of test tube. Formation of a blue coloured solution in acetic acid layer indicates the presence of cardiac glycosides (Junaid and Patil, 2020).

2.10.2 Kedee's Test

In this test, 4 mL extract evaporated to dryness is added to 1-2 mL methanol and then 1-2 mL alcoholic KOH followed by 3-4 drops of 1% alcoholic 3,5-dinitrobenzene and then heated. A disappearing violet colour shows the presence of Cardenolides (Kumar *et al*., 2018).

2.11 Test for Cardenolides

In this test, a sample extract is added to pyridine followed by addition of Sodium nitroprusside and 20% NaOH. A red colour, fades to brownish yellow shows the presence of cardenolides (Junaid and Patil, 2020)

2.11. 1 Bromine water Test

The sample plant extract is mixed with a few mL of bromine water. Formation of a yellow precipitate indicates the presence of cardenolides (Junaid and Patil, 2020)

2.11.2 Baljet test

In this test, 2mL of the extract is added to a few drops of Baljet's reagent. Formation of a yellow-orange colour shows the presence of cardenolides (Kumar *et al*., 2018).

2.12 Detection of Proteins and Amino Acid

In this test, 2mL of the sample filtrate is mixed 1 drop of 2% copper sulphate solution followed by addition of 1mL of 95% ethanol and KOH pellets. A pink colured solution in ethanolic layer confirms the presence of amino acids (Junaid and Patil, 2020)

2.13 Detection of Phlobatannins

2.13.1 Hydrochloric Acid Test

This involves addition of 2mL aqueous solution of the extract with 2mL 1% HCl and boil. An appearance of red precipitate indicates the presence of Phlobatannins (Njoku*et al.,* 2009).

2.14 Detection of Saponins

2.14.1 Foam Test

This test involves adding 0.5gm of the plant extract in 2mL water and vigorously shaken. Persistent foam for 10 minutes shows the presence of Saponins (Kumar *et al.,* 2013). Another way is by adding 20mL water in measuring cylinder followed by 50gm extract (vigorously shaken for 15 min.). Formation of 2cm thick layer of foam shows the presence of Saponins (Rauf *et al.,* 2013). Also, this can be achieved by adding 0.2gm of the plant extract with 5mL distilled water, shaken well and then heated. Appearance of creamy miss of small bubbles shows the presence of saponins (Ray *et al*., 2013).

2.14.2 $NaHCO_3$ Test

This involves addition of the plant extract with few mL sodium bicarbonate solution followed by addition of distilled water (vigorously shaken). Formation of Stable honeycomb like froth indicates the presence of Saponins (Kumar *et al*., 2013).

2.14.3 Olive Oil Test

This is done by adding an aqueous solution of the extract with 5mL distilled water, shaken vigorously and followed by a few drops of olive oil and the mixture is again shaken vigorously. Appearance of foam shows the presence of Saponins (Gul *et al*., 2017).

2.14.4 Haemolysis Test

This is done by adding a drop of fresh blood on glass slide and the plant extract. Formation of Zone of hemolysis shows the presence of Saponins (Bhatt and Dhyani, 2012; Sheel *et al.,* 2014).

2.15 Detection of Phytosterols

2.15.1 Salkowski's Test

This is done by adding the sample Filtrate with few drops of concentrated sulphuric acid (Shaken well and allowed to stand). Formation of a red colour in lower layer shows the presence of phytosterols (Tiwari *et al.,* 2011).

2.15.2 Libermann-Burchard's Test

A quantity 50 gm of extract is dissolved in 2mL acetic anhydride followed by addition of 1-2 drops of concentrated sulphuric acid (along the side of test tube). Formation of an array of colour change indicates the presence of phytosterols (Singh and Kumar, 2017).

2.15.3 Acetic Anhydride Test

A quantity 5mL of the plant extract is added to 2mL of acetic anhydride followed by addition of 2mL concentrated sulphuric acid. A Change in colour from violet to blue/green indicates the presence of phytosterols (Tiwari *et al*., 2011).

2.15.4 Hesse's response Test

This involves addition of 5mL of the aqueous solution of the sample extract with 2mL chloroform followed by addition of 2mL concentrated sulphuric acid. Formation of a pink ring / Red colour (in lower chloroform layer) shows the presence of phytosterols (Kumar *et al.,* 2018).

2.15.5 Sulphur Test

This involves addition of the extract solution with a pinch of sulphur powder. Phytosterols are present if the Sulphur sinks to the bottom (Pooja *et al*., 2016).

2.16 Detection of Cholesterol

This involves adding 2mL of the extract with 2mL chloroform followedby addition of 10 drops of acetic anhydride and 2-3 drops of concentrated Sulphuric acid. Formation of a red-rose colour shows the presence of Cholesterol (Jagessar, 2017).

2.17 Detection of Terpinoides

The procedure in this test involves mixing 2ml chloroform with 5mL of the sample plant extract, the mixture is evaporated on water bath followed by addition of 3mL concentrated Sulphuric acid (boiled on water bath). Formation of a grey-coloured solution indicates the presence of terpninoides (Gul *etal*., 2017).

2.18 Detection of Triterpinoid

2.18.1 Salkowski's Test

To the sample filtrate, a few drops of concentrated Sulphuric acid is added, shaken well and allowed to stand. Formation of a golden yellow layer (at the bottom) shows the presence of triterpinoids (Singh and Kumar, 2017).

2.19 Detection of Diterpene

The sample plant extract is dissolved in distilled water followed by addition of 3-4 drops of copper acetate solution. Formation of an Emerald green colour solution indicates the presence of triterpinoids (Pandey and Tripathi, 2014).

2.20 Detection of Lignins

2.20.1 Labat Test

The plant extract solution is mixed with gallic acid. Formation of an olive green colour shows the presence of lignins (Nanna *et al.,* 2013).

2.20.2 Furfuraldehyde test

The plant extract solution is combined with 2% furfuraldehyde solution. Formation of red color indicates the presence of lignins (Nanna *et al*., 2013).

2.21 Detection of Carotenoids

2.21.1 Carr-Price reaction

This method involves addition of 10mL extract which was evaporated to dryness with 2-3 drops of saturated solution of antimony trichloride in chloroform. Formation of blue-green colour eventually changing to red shows the presence of carotenoids (Jagessar, 2017).

2.22 Detection of Quinones

2.22.1 Alcoholic KOH test

This involves mixing 1ml plant extract with few mL alcoholic potassium hydroxide. Formation of a red to blue colour shows the presence of quinones (Singh and Kumar, 2017).

2.22.2 Conc. HCl Test

The sample plant extract is combined with concentrated HCl. A green colour formation shows the presence of quinones (Singh and Kumar, 2017).

2.22.3 Sulphuric Acid Test

This is done by taking 10mg of the sample plant extract and dissolved in isopropyl alcohol followed by addition of a drop of concentrated Sulphuric acid. Formation of precipitation shows the presence of quinones (Maria *et al.,* 2018).

2.23 Detection of Anthraquinones

2.23.1 Borntrager's Test

This is done by taking 10mL of 10% ammonia solution and mixed it with a few ml of the filtrate (shaken vigorously for 30 seconds). Formation of a pink, violet, or red coloured solution indicates the presence of Anthraquinones (Uma *et al*., 2017).

2.23.2 Ammonium Hydroxide Test

This is done by taking 10mg of the sample plant extract and dissolved in isopropyl alcohol followed by addition of a drop of concentrated ammonium hydroxide solution. Formation of red colour after 2 minutes shows the presence of Anthraquinones (Maria *et al*., 2018).

2.24 Detection of Anthocyanins

2.24.1 HCl Test

This is done by Nico 2mL of the plant extract with 2mL 2N HCl (+ few mL ammonia). Formation of a Pink-red solution which turns blue-violet after addition of ammonia shows the presence of anthocyanins(Obouayeba*et al.,* 2015; Savithramma*et al.,* 2011).

2.25 Detection of Carboxylic acid

2.25.1 Effervescence test

This is done by mixing 1mL plant of the extract with 1 mL sodium bicarbonate solution. Appearance of Effervescence shows the presence of carboxylic acid (Kumar *et al*., 2013).

2.26 Detection of Volatile Oils

2.26.1 Fluorescence test

10 mL of extract, filtered till saturation, exposed to UV light. Formation of a Bright pinkish fluorescence shows the presence of volatile oils (Mallhi*et al*., 2014).

2.27 Detection of Fixed Oils and Fats

2.27.1 Spot test/ Stain test

Little quantity of plant extract is pressed in between to filter papers. Oil stain on the paper shows the presence of fixed oils and Fats (Junaid and Patil, 2020).

2.27.2 Saponification Test

The sample extract is combined with a ffew drops of 0.5N alcoholic KOH anda few drops of phenolphthalein (Heated for 2hr.). Soap formation or partial neutralization of alkali shows the presence of fixed oils and Fats (Nanna *et al*., 2013).

2.28 Detection of Resins

2.28.1 Acetic Anhydride Test

This is done by mixing 1 mL of plant extract with Acetic anhydride solution and 1mL concentrated Sulphuric acid. Orange to yellow formation shows the presence of Resins (Singh and Kumar, 2017).

2.28.2 Turbidity Test

In this test, 1 mL of plant extract is dissolved in acetone, and then poured in distilled water. Formation of turbidity shows the presence of Resins (Kumar *et al.,* 2013).

2.28.3 Detection of Coumarins

In this test, 5gm moistened extract is taken in test tube, mouth of test tube is covered with 1N NaOH treated filter paper, heated for few minutes in water bath. Formation of a yellow fluorescence from paper under the UV light shows the presence of Coumarins (Singh and Kumar, 2017).

3. Reagents Preparation for Phytochemical Testing

3.1 Dragendroff's reagent preparation

Stock solution: 5.2gm Bismuth carbonate is added to 4gm sodium iodide followed by addition of 50mL glacial acetic acid, boiled for few minutes. After 12 hours, precipitated sodium acetate crystals are

filtered by sintered glass funnel; 40mL filtrate + 160mL ethyl acetate + 1mL distilled water, (stored in amber-coloured glass bottle).

3.2 Mayer's Reagent

Working solution: 10mL stock solution + 20mL acetic acid + distilled water to make final volume 100mL.

Solution A : 1.358gm mercuric chloride + 60mL distilled water

Solution B : 5gm potassium iodide + 10mL distilled water

Working solution: solution A + solution B + distilled water to make final volume 100mL

3.3 Hager's Reagent

Hager's reagent is a saturated aqueous solution of picric acid

3.4 Wagner's reagent

Wagner's reagent is a 1. 27gm iodine + 2gm potassium iodide + distilled water to make final volume 100mL

3.5 Barfoed's Reagent

Barfoed's reagent is a solution containing 30.5gm copper acetate + 1.8mL glacial acetic acid

3.6 Seliwanoff's Reagent

Seliwanoff's reagent is a solution containing 0.05 resorcinol + 100mL dilute HCl

3. 7 Benedict's Reagent

Solution A: 173gm sodium citrate + 100gm sodium carbonate + 800mL water, dissolve & boil to make solution clear

Solution B: 17.3gm of copper sulphate dissolved in 100mL distilled water

Working solution: Mix solution A and solution B

3.8 Fehling's solution

Solution A: 34.66gm copper sulphate + distilled water to make final volume 100mL.

Solution B: 173gm potassium sodium tartarate + 50gm NaOH + distilled water to make 100mL.

3.9 Million's Reagent

1gm mercury + 9mL fuming nitric acid + equal amount of distilled water (after completion of reaction) (Junaid and Patil, 2020).

Conclusion

Qualitative phytochemical screening techniques are also tools that are still essential in the initial exploration of medicinal plants. The techniques offer quick, easy, and inexpensive ways of screening significant categories of bioactive molecules that include alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, steroids, and phenolic compounds. Using classical chemical tests that rely on color changes, formation of precipitates or frothing reactions, and the researchers can rapidly determine the phytochemical profile of the plant extracts and find good candidates to proceed with in-depth

research. Even though qualitative techniques do not measure compounds or give detailed structural data, they constitute an important foundational part in the research of natural products. They inform the choice of the right solvents, extraction methods, and new techniques of analysis like chromatography and spectroscopy. Qualitative screening improves the productivity of phytochemical studies when applied in an organized and systematic way and coupled with adequate extraction methods, it minimizes resource and time wastage. Qualitative phytochemical screening is an indispensable preliminary step in the validation of traditional use of medicinal plants and in drug discovery and drug development programs. These methods, despite their shortfalls, still have a role to play, particularly in resource constrained environments, and are still part of ethno pharmacological research and natural products chemistry research.

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