WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.63
Volume 15, Issue 10, 576-605. Review Article ISSN 2277–7105

A REVIEW: EXTRACTION OF VALUABLE PHYTOCONSTITUENTS USING COLD-MACERATION METHOD WITH DIFFERENT POLAR AND NONPOLAR SOLVENTS FOR THEIR PHARMACOLOGICAL ACTIVITIES

Ranganatha Yadav H. R.[1], Gopinath S. M.[*]

[1]Department of Studies in Biotechnology, Davangere University, Shivagangothri, Davangere, Karnataka, India, 577007.

[2]Professor, Department of Studies in Biotechnology, Davangere University, Shivagangothri, Davangere, Karnataka, India, 577007.

Article Received on 15 April 2026,
Article Revised on 05 May 2026,
Article Published on 16 May 2026,

https://doi.org/10.5281/zenodo.20202050

***Corresponding Author**

Gopinath S. M.

Department of Studies in Biotechnology, Davangere University, Shivagangothri, Davangere, Karnataka, India, 577007.

How to cite this Article: Ranganatha Yadav H. R.[1], Gopinath S. M.[*]. (2026). A Review: Extraction of Valuable Phytoconstituents Using Cold-Maceration Method With Different Polar and Nonpolar Solvents For Their Pharmacological Activities. World Journal of Pharmaceutical Research, 15(10), 576–605.

ABSTRACT

The extraction of phytocompounds from medicinal plants is an important step in the production of plant-based medications. Cold maceration remains one of the most popular extraction processes due to its easy of use, low cost and ability to maintain thermolabile bioactive chemicals. This paper investigates the efficacy of the cold maceration method for extracting pharmacologically active phytochemicals utilising various solvents, including polar and non-polar solvents. The choice of solvent has a major impact on the yield, content and biological activity of the extracted phytoconstituent. Polar solvents like methanol, hydro-ethanol are useful in extracting phenolics, flavonoids, glycosides and tannins, but non-polar solvents like n-hexane and petroleum ether are more ideal for alkaloids, terpenoids and lipophilic compounds. This review also emphasises the importance of solvent polarity, maceration 0period, plant material-to-solvent ratio and ambient temperature in optimising the extraction process. Comparative pharmacological investigations reveal that cold maceration extracts exhibit a diverse range of biological activities, including antibacterial, antioxidant, anti-inflammatory, anticancer, and antidiabetic effects.

KEYWORDS: cold-maceration method, Methanol, Hydro-ethanol, N-hexane, Petroleum ether.

1. INTRODUCTION

1.1. Traditional Use of Medicinal Plants in Human Healthcare

The medicinal properties of plants have been used in China and India to treat various human health problems for centuries, dating back to pre-civilization times. The components of this plant have been utilized for a long time and possess a wide range of therapeutic properties. Approximately 30–50% of the indigenous people who live in remote and unreachable sections of the world's woods rely on medicinal plants for their basic medical needs (Maurya et al., 2022). Several factors result from the availability and cost of traditional medicine as a form of care in the primary healthcare system of resource-poor areas. Plants have been the main source of medicinal items used to treat human ailments since the dawn of humanity. Before recorded history, people were more or less aware of the properties of the plants around them since they had learned to recognize and use them for food, clothing, shelter and fuel.

1.2. Phytochemicals: Classification, Functions and Health Benefits

Phytochemicals, derived from the Greek term phyto, meaning plant, are physiologically active, naturally occurring chemical compounds present in plants These molecules have a greater positive impact on human health than macronutrients and micronutrients. Phytochemicals or phytocompounds have been categorized as primary and secondary components according to their function in plant metabolism.[2] Common sugars, amino acids, proteins, purines, pyrimidines and other primary ingredients that are directly involved in plant growth and development include amino acids, purines and pyrimidines of nucleic acids. Other plant compounds, including curcumines, saponins, phenolics, flavonoids, lignans, plant steroids and glucosides, which are indirectly involved in growth and development, are considered secondary ingredients.[3] Secondary phytocompounds have key qualities that can help prevent or treat several common ailments, even though they do not contain any necessary nutrients or are needed by the human body to support life.

1.3. Methods of extracting medicinal plants that priorities cold-maceration method

A study on medicinal plants[3] starts with pre-extraction and extraction techniques, which are important steps in the extraction of bioactive components from plant materials. Extraction is the process of separating the various components of a plant that are medicinally effective

using certain solvents and accepted practices. All extraction procedures have the same goal: to separate the plant's soluble metabolites from its insoluble cellular components. Some of the basic crude must be further processed. Maceration, infusion, percolation, decoction, Soxhlet extractors, and ultrasound-assisted extraction machines are the most frequently used extraction techniques**.** Among these methods, we chose the cold maceration technique.[4] In small research settings, maceration extraction is a widely used conventional method. The process of cold maceration, widely used in the production of red wine, has implications for logistics, finances, and product quality. The plant material was coarsely powdered and placed in a stoppered container with a solvent. The mixture was allowed to rest at room temperature for at least three days while being constantly stirred. The plant cell wall is intended to be weakened and ruptured during processing to release soluble phytochemicals.

1.4. The usual condition for maceration method.

Particle size	Coarse to medium powder to increase surface area without producing fines that complicate filtration
Solvent-to-solid ratio	Commonly 5:1 to 20:1 (mL/g) depending on matrix and solvent
Time	From 24 hr up to several days with intermittent agitation in some protocol use 24-72 hr
Temperature	20-25 ^{0}C
Agitation	Periodic shaking or mechanical stirring speeds equilibrium

1.5. Filtration and solvent removal

The extraction of phytocompounds is heavily dependent on the solvents used. Low toxicity, ease of evaporation at low heat, promotion of quick physiological absorption of the extract, preservation action and inability to induce the extract to complex or dissociate are all qualities of a suitable solvent in plant extractions. The choice of solvent is influenced by the amount of phytochemicals to be extracted, extraction rate, diversity of compounds extracted, diversity of inhibitory compounds extracted, ease of handling the extracts after extraction, solvent toxicity in the bioassay process and potential health risks posed by the extractants. The planned use of the extract affects the choice of solvent. Generally, there are two types of solvents: polar and nonpolar. Polar solvents generate a better yield of phytocompounds than non-polar solvents because of their larger dipole moments.

1.6. Solvent polarity: classification and common bioactives targets

Solvent polarity is the single most important factor determining which classes of phytochemicals are extracted. Common solvents used across polarity ranges.

Nonpolar solvents: n-hexane, petroleum ether. Targets: fixed oils, fatty acids, nonpolar terpenoids, sterols, carotenoids.

Polar solvents: Ethanol, methanol, water and methanol-water mixture. Targets: phenolic acids, flavonoids glycosides, tannins, alkaloids (depending on base/acid state), glycosides, polysaccharides.

Solvent choice is thus matched to the expected chemistry of the target phytoconstituents. Methanol and ethanol are widely used because they dissolve a broad range of polar constituents and are compatible with many analytical workflows; water extracts very polar materials but can co-extract sugars, proteins and large carbohydrate matrices.

Table I: Shows the polar and non-polar used for the extraction of active phytocompounds.

Polar and Non-polar Solvents			
1.Methanol	**2. Hydro-ethanol**	**1. n-hexane**	**2. Petroleum ether**
Polyphenols	Polyphenols		Alkaloids
Terpenoids	Terpenoids		Terpenoids
Saponins	saponins		
Lactones	Tannins		
Anthocyanins	Amino acids Carbohydrates Glycosides Flavonoids phenones		

1.7. Solvents polarity influences extraction yield and bioactivity

Many empirical research have found polarity-dependent variations in overall yield, phenolic/flavonoid content, and antioxidant or other bioactivities. In many plant matrices, highly polar solvents produce a higher mass yield (because they solubilise sugars and polar metabolites), whereas medium polarity solvents may concentrate antioxidant polyphenols more effectively; nonpolar solvents produce lower mass yields but concentrate lipophilic bioactives (essential oils, lipids) with distinct bioactivities (antimicrobial, anti-inflammatory). Several comparative studies and systematic reviews have found that utilising a panel of solvents (consecutive or parallel extraction) provides the most comprehensive phytochemical coverage and the highest possibility of linking certain pharmacologies to specific fractions.

Table II: Showing polar and non-polar solvents with their boiling point and dipole moment.

Sl No	Polar Solvents	Dipole	Boiling point	Non-polar solvents	Dipole Moment	Boiling point
1	Water	1.85	100	Petroleum ether	0	60^0C
2	Methanol	1.7	64.4	Hexane	0.08	68.70C
3	Ethanol	1.69	1.15	Chloroform	1.15	0C
4	Acetone	2.88	56	Chloro benzene	1.69	131.7
5	Ethyl acetate	1.88	40	Diethyl	1.15	34.5
6	2- propanol	1.58	82.5	Dichoromethane	1.6	39.6

2. Methanol was used as a solvent with cold-maceration method

Methanol is one of the most commonly used solvents for extracting phytocompounds due to its strong polarity and high extraction efficiency. It has been applied in various studies to obtain bioactive compounds with significant pharmacological properties.

The *Lantana camara* plant was studied to determine its potential antidiarrheal properties. Castor oil was used to induce diarrhea, while the plant's bioactive compounds were extracted using cold maceration with 80% methanol. The results showed that the methanol extract had strong antidiarrheal potential.[5] Similarly.[6] extracted bioactive compounds from *Newbouldia laevis* (Bignoniaceae) using methanol with cold maceration mwthod and the extract exhibited strong antihyperglycemic activity. According to.[7] *Combretum hypopilinum* has diverse therapeutic applications, including treatment of gastrointestinal disorders as well as cholagogic, diuretic and purgative conditions. Methanol extracts obtained through cold maceration revealed notable antidiarrheal activity and valuable phytochemicals.[8] investigated *Napoleona vogelii* Hook & Planch (Lecythidaceae) for antidiabetic and hypolipidemic effects in alloxan-induced diabetic models. Phytocompounds were extracted using 70% methanol via cold maceration. The extract exhibited hypoglycemic potential and contained important flavonoids, glycosides, tannins, and other quality phytochemicals. Likewise.[9] extracted phytocompounds from *Lophira lanceolata* (Ochnaceae) using methanol and cold maceration method to evaluate acute toxicity (LD50), antiplasmodial activity and antioxidant capacity. Antioxidant activity was assessed using DPPH, hydrogen peroxide scavenging, free radical scavenging and reducing ability assays, while antiplasmodial activity was tested using a rodent parasitemia model. The plant extract demonstrated significant antioxidant and antiplasmodial activity.

As per.[10] methanol and ethanol were used to extract bioactive compounds from *Musa paradisiaca* (banana) tepals via cold maceration. Methanol extracts (from tepal meat and skin) showed greater nitric oxide scavenging activity and anti-inflammatory properties than ethanol extracts.[11] extracted phytocompounds from *Callicarpa arborea* leaves with methanol using cold maceration and reported that the extracts exhibited antioxidant activity through different assays, suggesting their usefulness in preventing infections and degenerative diseases. Similarly,[12] extracted phytocompounds from *Solanum lycopersicum* (L.) using methanol and water. Methanol extracts displayed significant antibacterial activity in vitro compared to water extracts.[13] reported that methanol was used in cold maceration to extract bioactive compounds from *Anabasis articulata* stems. Antioxidant activity was tested using DPPH and radical scavenging assays, while anti-angiogenesis was assessed with a rat aorta ring test. The methanol extract showed notable antioxidant and anti-angiogenic activities compared to other solvents. According to[14] *Mikania micrantha* leaves were extracted with methanol via cold maceration, yielding high phenolic content and strong antioxidant activity. In another study,[15] extracted phytocompounds from the stems, barks and fractions of *Schwa infurthiona F. Haffm* (Ochnaceae) using methanol and other solvents. FRAP and DPPH assays revealed that methanol extracts exhibited stronger antioxidant activity than those from other solvents.

Cold maceration with methanol was also applied to *Euphorbia tirucalli*, along with petroleum ether, chloroform, and aqueous solvents.[16] Tested antifungal activity against *Aspergillus niger* and *Candida albicans* strains and found methanol and aqueous extracts to be the most effective. Similarly,[17] reported that people in northern regions consumed *Castanopsis costata* (C. costata) as an antioxidant dietary supplement. Methanol extracts of its leaves contained important phytochemicals and antioxidant properties comparable to vitamin C.

Two plants, *Solanum unigram* and *Solanum xanthocarpum*, were extracted using methanol and water via cold maceration.[18] evaluated their antimicrobial, antioxidant, antidiabetic, and hemolytic activities. Methanol extracts demonstrated higher free phenol content, strong inhibition of *E. coli*, and significant antioxidant activity.

Henna (*Lawsonia inermis*) leaves, traditionally used as dye, were extracted using cold maceration with methanol, water, and ethyl acetate. According to.[19] methanol and water extracts contained a notably high concentration of tannins, suitable for leather tanning and coloring. In another study,[20] used Soxhlet and cold maceration with several solvents to

extract *Moringa stenopetala* leaves (Moringaceae). Methanol and its aqueous fraction significantly reduced both central and peripheral pain and inflammation, indicating analgesic and anti-inflammatory effects.

The bioactive compounds of *Plectranthus amboinicus* leaves were extracted with methanol using cold maceration.[21] reported that these extracts had the highest phenolic and flavonoid content, strong DPPH scavenging activity, and superior antimicrobial properties against tested pathogens. Similarly,[22] extracted phytocompounds from *Euphorbia heterophylla* and *Tithonia diversifolia* using methanol via cold maceration. The methanol extracts showed significant inhibition of *Salmonella typhi* and *Pseudomonas aeruginosa*. According to,[23] methanol cold maceration of *Coldenia procumbens* Linn revealed phytochemical content and antioxidant activity through reducing power and hydrogen peroxide radical scavenging assays.

Bryophyllum pinnatum (Crassulaceae) was studied for antidiarrheal and antioxidant properties.[24] extracted its bioactive compounds with 80% methanol via cold maceration and found significant activity using DPPH and FRAP assays. Similarly,[25] extracted phytocompounds from *Agapanthus africanus* leaves with 80% methanol. Results confirmed acute toxicity and strong anti-inflammatory activity. In another study,[26] evaluated *Lupinus arboreus* extracts prepared with methanol for antioxidant and free radical scavenging activity.

After 72 hours of cold maceration, the methanol extract exhibited strong therapeutic value.[27] compared aqueous, methanol, ethanol, and acetone extracts of *Paederia foetida* obtained by cold maceration and found methanol to yield the highest phenolic content and strongest antioxidant activity in DPPH and ABTS assays.

According to.[28] methanol was used to extract phytochemicals from *Napoleona vogelii* leaves (Lecythidaceae). The extract showed strong antidiabetic efficacy in alloxan-induced diabetes and hypolipidemic activity. Similarly,[29] extracted bioactive compounds from *Citrullus colocynthis* using solvents such as butanol, methanol, chloroform, ethanol, and hexane.

Methanol and butanol extracts, being more polar, contained significant phytochemicals.[30] extracted *Thespesia populnea* phytocompounds with methanol, revealing the presence of carbohydrates, phenolics, flavonoids, alkaloids, and glycosides. Further studies confirmed methanol's effectiveness.[31] extracted *Garcinia kola*, *Kola auminate*, and *Kola vera* using

99.5% methanol via cold maceration and detected saponins, glycosides, volatile oils, steroids, and alkaloids.[32] studied aerial parts of *Semenovia suffruticosa* using methanol cold maceration. The extract showed antioxidant activity and tumor cell-specific cytotoxicity, confirmed through DPPH, agar disc, and MTT assays.[33] extracted *Pinus halepensis* and *Quercus ilex* L. using methanol cold maceration. The extracts demonstrated antioxidant, anti-hemolytic, and genoprotective properties due to high phenolic and flavonoid content.

Stachytarpheta indica (Verbenaceae) was extracted with methanol, and the extract confirmed the presence of alkaloids, saponins, tannins, anthraquinones, phenolics, and flavonoids.[34] It exhibited strong anti-inflammatory activity. Similarly,[35] evaluated *Boswellia dalzielli* (frankincense), extracted with methanol, followed by fractionation using n-hexane, chloroform, ethyl lactate, and n-butanol. The methanol extract showed significant antidiabetic activity in alloxan-induced diabetic rats and contained saponins, tannins, and terpenoids.

According to,[36] *Cardiospermum halicacabum* extracts obtained with methanol demonstrated strong antioxidant activity, confirmed through GC-MS analysis and DPPH assays.[37] extracted *Hibiscus sabdariffa* (Roselle) using methanol via cold maceration and found significant antioxidant activity, particularly in the calyx, leaves, and stem. Similarly.[38] compared extracts of *Fumaria officinalis* leaves prepared with methanol, petroleum ether, and chloroform. Methanol produced higher levels of phenolics and flavonoids, making it the most effective solvent. According to.[39] *Thuja occidentalis* extracts obtained with methanol displayed substantial phenolic and flavonoid content, strong antioxidant activity, and significant antimicrobial activity against resistant bacteria, comparable to antibiotics such as ofloxacin and amphotericin B.[40] studied *Rhododendron arboreum* (Ericaceae) flowers using 80% methanol and other solvents. Methanol extracts had higher total phenolic content (107.46 ± 8.74 mg/g RE) and greater antioxidant activity compared to ethanolic extracts.

Achyranthes aspera Linn bioactive compounds were extracted with methanol using cold maceration by.[41] The methanol extract showed notable antioxidant, antibacterial, and thrombolytic properties. According to,[42] *Fadogia cienkowskii* extracts obtained with methanol exhibited high total flavonoid and phenolic content (TFC and TPC) and strong antioxidant activity. Finally,[43] extracted phytocompounds from *Tinospora cordifolia* (gurjo) with methanol using cold maceration. The methanol extract demonstrated significant antioxidant and cytotoxic activity, as well as strong antimicrobial activity against *Streptococcus* strains.

3. Hydro-ethanol was used as a solvent with cold-maceration method

Water, ethanol, methanol, and acetone are the most commonly used solvents for preparing extracts with strong antioxidant activity. However, for food applications or when low toxicity is required, ethanol–water mixtures (hydro-ethanol) are considered the most suitable solvents.

These combinations are widely employed for extracting phenolic compounds from medicinal plants.

According to,[44] bioactive compounds from *Anabasis aretioides* Coss. & Moq. (Chenopodiaceae) were extracted using cold maceration and Soxhlet methods. Antioxidant activity was evaluated through different assays, and antibacterial activity was assessed using disc diffusion. Results showed that cold maceration extracts had higher antioxidant and antibacterial activity compared to Soxhlet extracts. As noted by,[45] solvents play a crucial role in phytoconstituent extraction. Hydro-ethanol, being a polar solvent, was used to extract bioactive compounds from *Citrullus colocynthis* (C. colocynthis). These extracts obtained through cold maceration demonstrated the ability to lower blood glucose levels.[46] reported that *Cardiospermum* L. phytocompounds were extracted using ethanol by the cold maceration method. The ethanol extract showed valuable antibacterial, antioxidant, analgesic, vasodepressant, and anti-inflammatory properties. A comparative study by[47] examined the yield and composition of *Bryophyllum* phytocompounds extracted by Soxhlet and cold maceration. The cold maceration method, using ethanol, produced a higher yield and a more diverse range of useful bioactive components compared to Soxhlet extraction. Similarly,[48] extracted phytocompounds from *Momordica cymbalaria* (Cucurbitaceae) using several techniques to determine phenolic and flavonoid contents, as well as antioxidant and antidiabetic activities. Cold maceration extracts showed stronger activity than Soxhlet extracts.

Phytochemicals have significant medicinal and pharmacological benefits. For instance, *Thymelaea hirsuta* was collected from different geographical regions, and its bioactive compounds were extracted by both Soxhlet and cold maceration methods. According to,[49] cold maceration extracts had the highest phenolic content, which was strongly correlated with antioxidant activity. In another study,[50] extracted phytocompounds from *Anisochilus carnosus* (L.F) against the gastric pathogen *Helicobacter pylori* using a water–ethanol solvent via cold maceration. The extract showed stronger inhibitory activity against *H. pylori* than Soxhlet extracts.

The medicinal and pharmacological properties of *Strobilanthes ciliatus* Nees have also been investigated. According to,[51] this plant possesses hepatoprotective, analgesic, and DNA-protective properties. Its bioactive compounds were extracted using ethanol through cold maceration, revealing the presence of alkaloids, phenols, flavonoids, and terpenes, with the extract showing greater analgesic activity than some standard drugs. Similarly,[52] extracted bioactive compounds from *Psidium guajava* leaves with 70% ethanol via cold maceration to test their antidiabetic potential. Using albino rats and alloxan induction, results showed that the ethanol extract had significant antihyperglycemic effects.

According to,[53] roots of *Saussurea lappa* were extracted with ethanol using cold maceration to evaluate analgesic and anti-inflammatory properties. The extract demonstrated considerable potential in both activities. Likewise, extracts of *Ocimum sanctum* and neem prepared via cold maceration were tested for antibacterial activity against gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria. Results confirmed strong antibacterial effects.[54] Furthermore,[55] isolated bioactive compounds from *Trachyspermum ammi* L. using cold maceration and reflux methods to test cytotoxicity, acetylcholinesterase (AChE) inhibition, and antioxidant activity. Cold maceration extracts contained the highest phenolic and flavonoid levels and exhibited stronger cytotoxicity and AChE inhibitory activity compared to reflux extracts.

In another study,[56] screened phytocompounds from *Muntingia calabura* leaves collected in Colombia. Extraction was performed using 96% ethanol via cold maceration, and the leaves were found to contain several valuable phytochemicals. Similarly,[57] examined *Psidium guajava* (Myrtaceae), a plant widely used in treating inflammation, diabetes, hypertension, wounds, and fever. Phytochemicals in the leaves were extracted with various solvents through cold maceration. The results confirmed that *P. guajava* leaves contained highly valuable bioactive compounds.

4. Petroleum ether was used as a solvent with cold-maceration method

Petroleum ether is a non-polar solvent widely used to extract phytocompounds. In pharmacognosy laboratories, it is commonly applied for plant extraction and separation through chromatography. However, residues of petroleum ether may remain in herbal extracts if not fully removed, which can potentially affect their pharmacological properties.

According to,[58] petroleum ether extracts of fenugreek obtained through cold maceration were evaluated for anti-inflammatory activity using rats with carrageenan- and formaldehyde-induced paw edema. Gas–liquid chromatography was used for analysis, and the petroleum ether extract showed significant anti-inflammatory effects. The study by.[59] aimed to evaluate anti-angiogenic and antioxidant activities by extracting *Anabasis articulata* stems with various solvents, including petroleum ether, chloroform, and methanol, using cold maceration. Results revealed higher percentages of anti-angiogenic and antioxidant activity in polar solvents compared to non-polar solvents like petroleum ether.

Orthosiphon stamineus Benth leaves were extracted with methanol, petroleum ether, chloroform, and water using cold maceration.[60] All solvent extracts exhibited anti-angiogenic and antioxidant activity, but methanol showed the strongest effects due to its polarity. Similarly,[61] extracted bioactive compounds from *Lantana camara* L. leaves with petroleum ether using cold maceration to assess antioxidant activity and oxidative stability. Antioxidant stability was determined using the Rancimat test. When rich in terpenes, petroleum ether extracts displayed significant antioxidant activity (IC50 value 13 µg/ml) and offered 74.83% greater protection against oxidation compared to standard drugs.

To evaluate antimicrobial activity,[62] tested petroleum ether extracts of *Platychaete aucheri* (Boiss) against *Pseudomonas aeruginosa* PTCC 1430, *Staphylococcus aureus* PTCC 1431, *Bacillus cereus* PTCC 1247, and *Candida albicans* PTCC 5027. Results showed that petroleum ether and chloroform extracts had stronger bactericidal activity than methanol extracts. According to,[63] petroleum ether was also used to extract *Cleome viscosa* Linn using cold maceration, along with ethyl acetate, methanol, and aqueous solvents. Biochemical tests indicated that petroleum ether extracts showed better wound-healing activity than other solvents.

In another study,[64] extracted bioactive compounds from *Amaranthus viridis* and *Amaranthus tricolor* herbs using petroleum ether, chloroform, and ethanol through cold maceration. TLC fingerprint profiling confirmed that petroleum ether and chloroform extracts contained valuable phytochemicals with nutraceutical potential. Similarly, *Achyranthes aspera* roots extracted with petroleum ether demonstrated antibacterial activity, as reported by.[65]

According to,[66] petroleum ether extracts of the whole plant *Bergia suffruticosa* were tested for antioxidant, cytotoxic, and anticancer properties. The anticancer activity was measured

using the SRB test against breast carcinoma (MCF-7), prostate carcinoma (PC3), and colon carcinoma (HCT-116) cell lines. The petroleum ether extract exhibited strong antioxidant activity (IC50 = 89.01 µg/ml) and effective anticancer action.

Furthermore,[67] extracted *Bixa orellana* Linn leaves with petroleum ether via cold maceration. Screening confirmed the presence of valuable phytochemicals, and the petroleum ether extract demonstrated the strongest antibacterial activity against *S. aureus*, with an inhibition zone of 5.6 mm. It also showed general antimicrobial potential. According to,[68] *Carica papaya* plant parts were extracted with petroleum ether and other solvents to evaluate antimicrobial activity. Results revealed that petroleum ether extracts had the largest inhibition zone (22 ± 0.0 mm) against *S. aureus*, while *E. coli* showed minimal inhibition (0.13 ± 0.00 mm) and *K. pneumoniae* had no inhibition.[69] extracted phytocompounds from roots and shoots of *Phyllanthus debilis* Klein ex Willd using both polar and non-polar solvents (petroleum ether, chloroform, acetone, ethanol, and water) via cold maceration. The study found that polar solvents exhibited much higher antioxidant activity in the DPPH assay compared to non-polar solvents. Finally,[70] assessed antimicrobial activity of *Cucumis pustulatus* and *Vernonia schimperi* using extracts prepared with petroleum ether, methanol, and distilled water through cold maceration. Results indicated that extracts from polar solvents possessed stronger antimicrobial activity and more valuable phytochemicals compared to petroleum ether.

5. N-hexane was used as a solvent with cold-maceration method

[71]used cold maceration to extract bioactive compounds from *Moringa oleifera* roots using n-hexane as the primary solvent. Antibacterial activity was evaluated against clinically isolated *Staphylococcus aureus* using agar-dilution testing. Gas chromatography–mass spectrometry (GC-MS) was performed to analyze the extract and identified 45 compounds.

The major constituents included 9,12-octadecadienoic acid (41.08%), 2-chloroethyl linoleate (41.08%), n-hexadecanoic acid (17.35%), 12-octadecadienoic acid (8.55%), stigmasterol (4.44%), ergost-22-en-3-one (4.44%), and campesterol (3.46%), all of which exhibited significant antibacterial potential.

To determine total phenolic and flavonoid contents,[72] extracted phytocompounds from *Voacanga africana* using methanol, and further fractionated them into n-hexane, ethyl acetate, and n-butanol fractions via cold maceration. Results indicated that the n-hexane

fraction had the highest total phenolic content (GAE: 116.60 ± 7.95 mg/g) and flavonoid levels. In another study,[73] extracted bioactive compounds from *Thymelaea hirsuta* L. aerial parts using Soxhlet and cold maceration with n-hexane, ethyl acetate, and methanol. Antioxidant activity was assessed using DPPH and ABTS assays. Methanol cold-maceration extracts exhibited higher levels of bioactive compounds and stronger antioxidant activity than n-hexane and ethyl acetate extracts.

According to,[74] phytocompounds from *Senna italica* were extracted with n-hexane by cold maceration and analyzed using GC-MS, which revealed valuable constituents such as phytol (3,7,11,15-tetramethyl-1-hexadecen-1-ol) and 1,2-benzenedicarboxylic acid. Similarly,[75] extracted phytocompounds from the roots and stem bark of *Xylopia aethiopica* using n-hexane, n-butanol, and petroleum ether. Results showed that n-hexane extracts exhibited weaker antibacterial activity compared to petroleum ether extracts.

Chromophora senegalensis was extracted using cold maceration with n-hexane, ethyl acetate, methanol, and water.[76] Among the solvents, the n-hexane extract contained the lowest alkaloid levels and showed the least suppression of *Plasmodium falciparum*. Likewise,[77] studied *Diospyros blancoi*, *Phoenix dactylifera*, and *Morus nigra* for their antibacterial potential against oral pathogens. Extracts were prepared with n-hexane, chloroform, methanol, ethyl acetate, and water. The n-hexane extracts contained the lowest phytochemical levels and showed the weakest antibacterial activity.

According to.[78] *Fagonia indica* Burm. f. phytocompounds were extracted using water, methanol, chloroform, and n-hexane, and tested for antioxidant and antidiabetic activities.

While several phytochemicals were detected, chloroform and methanol extracts produced substantial results, whereas n-hexane extracts showed minimal activity. Similarly,[79] extracted phytocompounds from *Mentha piperita* (peppermint) leaves using n-hexane, acetone, dimethyl ether, petroleum ether, chloroform, ethanol, and water. Phytochemicals such as alkaloids, flavonoids, glycosides, terpenoids, saponins, and phenols were detected in all extracts except n-hexane.

As per.[80] bioactive compounds from *Cordia sebestena* L. were extracted by cold maceration with n-hexane, water, and 70% methanol. TLC profiling revealed 5, 7, and 2 distinct spots for n-hexane, dichloromethane, and methanol extracts, respectively, suggesting different

phytochemical compositions. Finally,[81] reported that phytocompounds from *Stephania abyssinica* were extracted using cold maceration with methanol, n-hexane, chloroform, and ethyl acetate to evaluate antimalarial activity against *Plasmodium berghei* in mice. Results showed that n-hexane was the least effective chemopreventive agent, while chloroform extracts exhibited the highest chemoprevention (55.80%).

Table IV: Showing the Pharmacological properties of medicinal plants extracted using different solvents with cold maceration method.

Plant name	Plant part	Solvents	Method of extraction	Active phyto compounds	Experimental design	Pharmacological properties	Reference
lantana camara	Root	Methanol extract	Cold-maceration method	Al, Fl, Ste, Sa, Ta, Ter	*In-vitro*	Anti hyperglycemic anti-diarrheal activities	[05]
New bouldia laevis	Leaf	Methanol extract	Cold-maceration Method		*In-vivo* (rats)	Anti-hyperglycemic activity	[06]
Combretum hypopilimum	Stem bark	Methanol extract	Cold-maceration Method	Car, Al, Phe, Ste, Ter, Sap, Fla, Tan	*In-ivtro*	Anti-diarrheal activity	[07]
Napoleona vogelii hook and planch	leaf	70% methanol	Cold-maceration Method	Fla, Sap, Gly, Tan, Anthraquinones.	*In-vivo (rats)*	Antidiabetic, Hypolipidemic activity	[08]
Lophira lanceolata	Fresh leaf	Methanol extract	Cold-maceration method	Fla, Alk, Oils, Gly, Carbs, Acids, Ter	*In-vivo (rodent-mice) & In-vitro*	Anti-plasmodial activity, anti-antioxidant activity (in-vitro)	[09]
Musa paradisiaca	Tepal, peel, pulp	Methanol extract	Cold-maceration Method	Fla, Sap, Phe	*In-vitro*	Anti-inflammatory, Nitric oxide scavenging activity	[10]
Callicarpa arborea	leafs	Methanol extract	Cold-maceration Method	Phe, Fla	*In-vitro*	Anti-oxidant activity	[11]
Solanum lycopersicum	Fresh fruit	Methanol extract	Cold-maceration Method	Al, Fla, Gly, Sap, Tan, Ste, Phl, Ter, Tan	*In-vitro*	Anti-microbial activity	[12]
Anabasis atriculata	stem	Methanol extract	Cold - maceration method		*In-vitro*	Antiangiogenesis Antioxidant activity	[13]

Makania micrantha	leafs	Methanol extract	Cold-maceration method	Phe, Fla	*In-vitro*	Anti-oxidant activity	[14]
schweinfurthian a F.Hoffm	Leaf, Stem-bark	Methanol extract	Cold-maceration method		*In-vitro*	Anti-oxidant activity	[15]
Euphorbia tirucalli	Stem Root	Methanol extract	Cold-maceration method	Alk, Tan, Sap, Fla	*In-vitro*	Anti-fungal activity Anti-microbial activity	[16]
castnopsis costata	Leaf	Methanol extract	Cold-maceration method	Alk, Fla, Gly, Anthra,Gly, Tan, Tri ter.	*In-vitro*	Anti-oxidant activity	[17]
Solanum Unigram & solanum xanthocarpum	Leaf fruit	Methanol extract	Cold-maceration method		*In-vitro*	Antioxidant, Antimicrobial, Antidiabetic and Hemolytic activities	[18]
Lawsonia inermis	leaves	Methanol extract	Cold-maceration method	Tan, Sap, Fla, Alk, Gly, Phe, Anthra	*In-vitro*		[19]
Moringa stenopetala	leaves	Methanol extract	Cold-maceration method	Fla, Tan, Ter, Sap, Ste, Gly, Alk	*In-vivo (swiss albino mice)*	Anti-inflammatory, Analgesic activity	[20]
plectranthus amboinicus	leaves	Methanol Extract	Cold-maceration method	tetracontane (16.6%), squalene (15.6%), tetrapentacontane (13.7%), and Phytol (12.9%).	*In-vitro*	Anti-oxidant activity Antimicrobial activity	[21]
Euphorbia heterophylla & Tithonia diversifolia	leaves	Methanol extract	Cold-maceration method		*In-vitro*	Anti-bacterial activity	[22]
Coldenia procumbens	leaves	Methanol Extract	Cold-	Ste, Fla, Alk, Phe,Gly	*In-vitro*	Anti-oxidant activity	[23]

			maceration method				
bryophyllum pinnatum	leaves	Methanol extract	Cold-maceratio Method		*In-vitro*	Anti-diarrheal and antioxidant activity	[24]
Allophylus africanus	leaves	80% hydro-Methanol Extract	Cold-maceration Method	Car, Tan, Ste, Tri, Fla, Alk, Cariac glycoside	*In-vivo (Beauv in rat)*	Anti-inflammatory activity	[25]
Lupinus arboreus	Leaves	Methanolic extract	Cold-maceration Method		*In-vitro*	Anti-oxidant activity	[26]
Napoleona vogeliis	Leaves	Methanol extract	Cold-maceration method		*In-vitro*	Anti-diabetic activity	[27]
Paederia foetida	leaves	Methanol extract	Cold-maceration Method				[28]
Cucurbit Citrullus colocynthis	Leafstem, Root, Tendrils Fruit pulp	Methanolic extract	Cold-maceration Method	Alk, Gly, Tan, Fla,Ste, Phe	*In-vitro*		[29]
Thespesia populnea	leaves	Methanol extract	Cold-maceration Method	Car, Phe, Fla, Alk, Gly, Sap, Ste, Pro	*In-vitro*	Anti-inflammatory activity	[30]
Garcinia kola, kola acuminate, kola vera	Kola nuts	Methanol extract	Cold-maceration method	Sap, Gly, Alk, Sap gly, Tan, Ste			[31]
Semenovia suffruticosa	Aerial parts	Methanol extract	Cold-maceration	Z-β-ocimene (25.1%), linalool (17.8%) and β-bisabolol (13.3%)	*In-vitro*	Cytotoxic, anti-oxidant, antibacterial activity	[32]
Quercus ilex Pinus halepensis	Root, Bark Young ovulate	Methanol extract	Cold-maceration Method	Flavonoids:- Catechin and phenolic acids (4-hydoxybenzoic,	*In-vitro*	Anti-oxidant, anti-hemolytic, genoprotective effect	[33]

				caffeic, coumaric, ferulic and gentisic acids			
Stachytarpheta indica	leaves	Methanolic extract	Cold-maceration Method	Alk, Sap, Car, Car, Gly, Ter, Tan, Anth, Phe, Fla	*In-vitro*	Anti-inflammatory activity	[34]
Boswellia dalzielli	Fresh leaf	Mathanolic extract	Cold-maceration method	Alk, Cardiac glycoside, Fla, Sap, Tan, Ter	*In-vivo*	Anti-diabetic activity	[35]
Cardiosperm halicacabum	Leaves	Methanolic extract	Cold-maceration method	Gly, Tan, Fla, Ter, Phe, Sap	*In-vitro*	Anti-oxidant activity	[36]
Hibiscus Sabdariffa	Leaf stem calyx of roselle	Methanolic extract	Cold-maceration Method	Phe, Fla, Alk, Antho, Anthra, Cardiac glycosides, Ter, Tan, Ste, Sap	*In-vitro*	Antioxidant activity	[37]
Fumaria officinalis	leaf	Methanolic extract	Cold-maceration method	Car, Gly, Alk, Fla, Tri, Ste	*In-vitro*	Antioxidant activity	[38]
Thuja occidentalis	leave	Methanolic extarct	Cold-maceration Method	Alk, Gly, Phe, Flav, Tan	*In-vitro*	Anti-microbial and Anti- oxidant activity.	[39]
Rhododendron arboreum	leaf	Methanolic extract	Cold-maceration Method	Phe, Alk,	*In-vitro*	Antioxidant activity	[40]
Achyranthes aspera	Stem leaves	Methanolic extract	Cold-maceration Method		*In-vitro*	Anti-oxidant activity, ant- inflammatory, anti-microbial and thrombolytic activity	[41]
Fadogia cienkowskii scheinf	leaf	Methanolic extract	Cold-maceration method	Alk, Gly, Tan, Sap, Fla, Ter	*In-vitro*	Antioxidant activity	[42]

Tinospora cordifolla		Methanolic extract	Cold-maceartion method	Alk, coumarin saponins, Gly, Reducing sugar, Triterpenes.	*In-vitro*	Anti-microbial activity, Anti- oxidant and Cytotoxicity activity	[43]
Anabasis aretoides coss & Moq	Aerial	Hydro- ethanolic extract	Cold-maceration method	Cathechic tannins, Sap, Ste.	*In-vitro*	Antibacterial anti-oxidant activity	[44]
Citrullus colocynthis	Fruit	Hydro-ethanolic extract	Cold-maceration method		*In-vivo*	Ant-diabetic activity	[45]
Cardiosperm	Leaf	Hydro- ethanolic Extract	Cold-maceration method	Glycosides, Tan, Fla, Phe, Sap	*In-vitro*	Anti-oxidant activity	[46]
Bryophyllum pinnatum	Aerial part & leaves	Hydro- ethanolic extract	Cold-maceration Method	iperazine, 1-(2-adamantyl)-4-benzoyl,	*In-vitro*		[47]
Momordica cymbalaria	leaves	Hydro- ethanolic extract	Cold-maceration method	Fla, Phe	*In-vitro*	Anti-oxidant, anti-diabetic activity.	[48]
Thymelaea hirsuta	aerial parts	Hydro- ethanolic extract	Cold-maceration Method	Phenolic compounds	*In-vitro*	Anti-oxidant activity	[49]
ANISOCHILUS CARNOSUS	Leaf	Hydro- ethanolic extract	Cold-maceration method		*In-vitro*	Anti-microbial activity	[50]
Strobilanthes ciliatus	Stem	Hydro ethanolic extract	Cold-maceration Method	Fla, Phe, Tan,Ste,Tri	*In-vivo*	Anti-Analgesic activity	[51]
Psidium guajava	leaf	Hydro- ethanolic extract	Cold-maceration method		*In-vivo*	Anti-hyperglycemic activity	[52]
Saussurea lappa	Root	Ethanolic extract	cold-		*In-vitro In-vivo*	Analgesic and anti-	[53]

			maceration method			inflammatory activity,	
Ocimum sanctum And azadirachta indica	leaves	Ethanolic extract	Cold-maceration Method	Tan, Sap, Fla, Anthraquinone, Gly, Ste	*In-vitro*	Anti-microbial activity	[54]
Trachyspermum ammi L.	Fruits	Ethanolic Extract	Cold-maceration Method	Phen, Flav	*In-vitro*	Cytotoxic, anti-acetylcholinesterase, anti- oxidant activities	[55]
Muntingia calabura	Fruits Brances flowers	Ethanolic extract	Cold-maceration method	sesquiterpene and a lignin (α-eudesmol and sesamin), hexatriacontane, scopolin and flavonoid 3, 5, 7,3′,4′ Pentahidroxiflavona (quercetin)	*In-vitro*	Screening of phytocompounds	[56]
Psidium guajava	leaves	Hydro ethanolic extract	Cold-maceration Method				[57]
Fenugreek	Seed powder	Petroleum ether extract	Cold-maceration method	Oleic, linoleic, and linolenic acids.	*In-vivo*	Anti-inflammatory,	[58]
anabasis articulata	Stem	Petroleum ether extract and methanol extract	Cold-maceration Method		*In-vitro*	Anti-angiogenic, anti-oxidant activity.	[59]
Orthosiphon stamineus benth	Leaves	Petroleum ether, methanol, chloroform extract	Cold-maceration method		*In- vitro*	Anti- Angiogenic, Anti-oxidant activity.	[60]
Latana camara	Leaves	Petroleum ether extract	Cold-maceration method	Pentacyclictrite rpenoids, steroid, Iridoid glycosides, Flavonoids aglycones,	*In-vitro*	Anti-oxidant and oxidative activity.	[61]

				polyphenols			
Platychaete aucheri	Aerial parts	Petroleum ether and chloroform extract	Cold-maceration method		*In-vitro*	Anti-microbial activity.	[62]
cleome viscoea	Seeds	Petroleum ether extract	Cold-maceration method	Ter, Alk, Phe, Fla, Sap	*In-vivo*	Wound healing activity	[63]
Amaranthus viridis and Amaranthus tricolor	Different parts	Chloroform and petroleum ether extract	cold-maceration method	Gly, Alk, Fla, Sap, Res, Phy		Nutraceutical potential	[64]
Achyranthus aspera	Root	Petroleum ether, chloroform extract	Cold-maceration method	Pro, Ste	*In-vitro*	Antimicrobial activity.	[65]
Bergia suffraticosa	Whole plant	Petroleum ether extract	Cold-maceration method		*In-vitro* *In- vivo*	Antioxidant and cytotoxicity, anticancer cancer activity	[66]
Bixa orellana .	Leaves	Petroleum ether extract	Cold-maceration Method	Alk, Tri, Ste, Tan, Sap, Fla, Phe, Pro, Gly, Sug, Fats and oils	*In-vitro*	Anti-bacterial activity	[67]
Caric papya	leaves	Petroleum ether extract	Cold-maceration method	Alk, Sap, Fla, Ste, Car, Gly, Tan	*In-vitro*	Anti-microbial activity	[68]
Phyllanthus debilis kleinex	Root Shoot	Petroleum Ether extract	Cold-maceration method	Quinone, cardioglycoside	*In-vitro*	Antioxidant activity	[69]
Cucumis pustulatus And vernonia schimperi			Cold-maceration method				[70]

CONCLUSION

The cold maceration method remains a widely utilized and effective technique for the extraction of bioactive phytoconstituents from various plant materials, especially when thermolabile compounds are of interest. The efficiency of this method is significantly influenced by the polarity of the solvents used, which determines the type and yield of phytochemicals extracted. Polar solvents such as methanol, ethanol and water have demonstrated high efficacy in extracting phenolics, flavonoids, glycosides and alkaloids compounds typically associated with potent antioxidant, antimicrobial, anti-inflammatory and anticancer activities. On the other hand, non-polar solvents like hexane, petroleum ether and chloroform are more suitable for isolating lipophilic constituents such as terpenoids, fatty acids, and certain essential oils, which also possess noteworthy pharmacological effects.

This review emphasizes that the choice of solvent is critical and should be tailored based on the target compound class and intended pharmacological application. Furthermore, the cold maceration technique offers advantages such as simplicity, cost-effectiveness and preservation of heat-sensitive compounds, making it particularly suitable for both laboratory-scale studies and traditional medicinal preparations. In conclusion, cold maceration with appropriate solvent selection remains a valuable tool in natural product research and pharmacognosy, contributing significantly to the discovery and development of plant-based therapeutic agents.

REFERENCES

1. Maurya, D., Kumar, T., Adhikari, C., Kumar, A., & Bishwas, A. J. (2022). Medicinal plants and their traditional knowledge in past history and future perspective. In D. Das (Ed.), Medicinal plants and traditional knowledge in the Indian subcontinent, 34–45. Shashwat Publication, Bilaspur.
2. Yadav, R. N. S., & Agarwala, M. (2014). Phytochemical analysis of some medicinal plants. Journal of Phytology, 6(1): 10–14.
3. Krishnaiah, D., Sarbatly, R., & Bono, A. (2009). Phytochemical antioxidants for health and medicine: A move towards nature. Biotechnology and Molecular Biology Reviews, 4(4): 97–104.
4. Azwanida, N. N. (2015). A review on the extraction methods used in medicinal plants: Principle, strength, and limitation. Medicinal & Aromatic Plants, 4: 196.
5. Handa, S. S., Khanuja, S. P. S., Longo, G., & Rakesh, D. D. (2008). Extraction

technologies for medicinal and aromatic plants (1st ed., No. 66).

6. Mengistu, G., Engidawork, E., & Nedi, T. (2015). Evaluation of the antidiarrhoeal activity of 80% methanol extract and solvent fractions of the leaves of Lantana camara Linn (Verbenaceae) in mice. Ethiopian Pharmaceutical Journal, 31: 107–120.
7. Chinyelu, C. O., Peter, A. A., Chukwuenmeka, S. N., Theophine, C. O., & Michel, K. T. (2015). Antihyperglycemic studies on the leaf extract and active fractions of Newbouldia laevis (Bignoniaceae). Pharmacology & Pharmacy, 6: 518–532.
8. Ismail, H. A., Hassan, H. S., Ilyas, M., & Sadam, A. A. (2021). Evaluation of phytochemical and anti-diarrheal activity of methanol stembark extract of Combretum hypopilinum Diels (Combretaceae). Nigerian Journal of Pharmaceutical Research, 17(1): 45–52.
9. Owolabi, O. J., Inninh, S. O., Anaka, O. N., & Iyamu, O. A. (2014). Antidiabetic and hypolipidemic effects of methanol leaf extract of Napoleona vogelii (Lecythidaceae) Hook & Planch on alloxan–induced diabetes mellitus in rats. Tropical Journal of Pharmaceutical Research, 13(11): 1903.
10. Onyeto, C. A., Akash, P. A., Nworu, C. S., Okoye, T. C., Okorie, N. A., Mbaoji, F. N., Nwabunike, I. A., Okumah, N., & Okpara, O. (2014). Anti-plasmodial and antioxidant activities of methanol extract of the fresh leaf of Lophira lanceolata (Ochnaceae). African Journal of Biotechnology, 13(16): 1731–1738.
11. Ahmad, B. A., Mahadeva Rao, U. S., & Mohd, K. S. (2016). In vitro nitric oxide scavenging and anti-inflammatory activities of different solvent extracts of various parts of Musa paradisiaca. Malaysian Journal of Analytical Sciences, 20: 1191–1202.
12. Inaotombi, L. D., Lallianchhunga, M. C., Lalmuanthanga, C., Lalchandama, C., Subudhi, P. K., & Ayub, M. A. (2016). In vitro antioxidant activity of methanolic extract of Callicarpa arborea leaves. World Journal of Pharmaceutical Research, 5: 2097–2102.
13. Umar, M., Zubairu, A., Hamisu, H. S., Mohammed, I. B., Oko, J. O., Abdulkarim, I. M., Salisu, A., Yaya, A. A., & Aliko, A. A. (2016). Evaluation of phytochemical and in vitro antimicrobial effects of Solanum lycopersicum Linn. (Tomato) on oral thrush and human cariogenic pathogens. JAMPS, 11(4): 1–9.
14. Abdulsahib, W. K., Abdulkareem, H. A., Qasim, B. J., & Sahib, H. B. (2016). Antiangiogenesis and antioxidant effect of Anabasis articulata stems extracts. International Journal of Pharmaceutical Sciences Review and Research, 41(2): 88–94.
15. Devi, L. I., Lallianchhunga, M. C., Ali, A. M., Lalchhandama, C., & Lalmuanthanga, C. (2016). Antioxidant activity of methanolic extract of Mikania micrantha leaves. World

Journal of Pharmaceutical Research, 5: 879–886.

16. Sultan, S., Kimaro, C. C., & Amri, E. (2016). Antifungal activity and phytochemical screening of different solvent extracts of Euphorbia tirucalli Linn. JABB, 7(1): 1–9.
17. Alkandahri, M. Y., Nisriadi, L., & Salim, E. (2016). Secondary metabolites and antioxidant activity of methanol extract of Castanopsis costata leaves. Pharmacology and Clinical Pharmacy Research, 1. ISSN: 2527-7322.
18. Sorna, R. S. A., Hariprasanth, R. J., Siddharth, M. P., Gobinath, M., Rajukutty, C. (2016). Evaluation of the antioxidant, antimicrobial, antidiabetic and hemolytic activity of organically grown Solanum nigrum and Solanum xanthocarpum. International Journal of Current Pharmaceutical Review and Research, 7(5): 296–299.
19. Danzarami, D., Umar, M., Akafyi, D. E., Oko, J. O., Yusuf, I. S., Abdulkarim, M. L., & Adamu, R. (2016). Phytochemical screening and chromatographic analysis of henna (Lawsonia inermis) plant obtained from Zaria Kaduna.
20. Tamrat, Y., Nedi, T., Assefa, S., Tiklehaymanot, T., & Shibeshi, W. (2017). Anti-inflammatory and analgesic activities of solvent fractions of the leaves of Moringa stenopetala Bak. (Moringaceae) in mice models. BMC Complementary and Alternative Medicine, 17: 473.
21. Swamy, M. K., Arumugam, G., Kaur, R., Ghasemzadeh, A., Mohd, M., Yusoff, Sinniah, U. R. (2017). GC-MS based metabolite profiling, antioxidant and antimicrobial properties of different solvent extracts of Malaysian Plectranthus amboinicus leaves. Evidence-Based Complementary and Alternative Medicine, 2017.
22. Oso, B. A., & Ogunnusi, T. A. (2017). Antibacterial activity of methanolic extracts of Euphorbia heterophylla and Tithonia diversifolia against some microorganisms. EJMP, 20(3): 1–8.
23. Suvarna, C. M., Santosh, B., Sireesha, C., & Durga, C. S. (2017). Phytochemical screening and in-vitro antioxidant activity of methanolic leaf extract of Coldenia procumbens Linn. World Journal of Pharmaceutical Research, 6: 839–845.
24. Onoja, S. O., Ihejirika, G. Q., Nwankudu, O. N., Omesh, Y. N., & Ezeja, M. I. (2018). Antidiarrheal and antioxidant activities of methanol extract of Bryophyllum pinnatum leaf harvested from South-Eastern Nigeria in mice. Journal of Pharmaceutics, 10: 1–6.
25. Ibrahim, F. S., Mohammed, Z., Nuhu, A., Shehu, S., & Ilyas, N. (2018). Acute toxicity and anti-inflammatory activity of hydromethanol leaves extract of Allophylus africanus Beauv in rats. J Herbmed Pharmacol, 7(2): 119–123.
26. Ohadoma, S. C., & Eban, L. K. (2018). Antioxidant and free radical scavenger effects of

methanol leaf extract of Lupinus arboreus. European Journal of Biomedical and Pharmaceutical Sciences, 5: 70–73.

27. Ojha, S., Raj, A., Roy, A., & Roy, S. (2018). Extraction of total phenolics, flavonoids and tannins from Paederia foetida L. leaves and their relation with antioxidant activity. Pharmacognosy Journal, 10(3): 541–547.
28. Owolabi, O. J., Inninh, S. O., Anaka, O. N., & Iyamu, O. A. (2018). Antidiabetic and hypolipidemic effects of methanol leaf extract of Napoleona vogelii (Lecythidaceae) Hook & Planch on alloxan–induced diabetes mellitus in rats. Tropical Journal of Pharmaceutical Research, 13(11): 1903–1909.
29. Ramakrishna, D., Suvarchala, V., Chaitanya, G., & Shasthree, T. (2019). Preliminary phytochemical screening of a medicinally important cucurbit Citrullus colocynthis (L.) Schard. Research Journal of Chemistry and Environment, 23(11).
30. Jayasri, A. Dr., Prasad, P. E., Padmaja, K., Kalakumar, B., Gnanaprakash, M., Adilaxmamma, K., & Prasad, T. N. V. K. V. (2019). Phytochemical analysis of methanolic leaf extract of Thespesia populnea. Journal of Pharmacognosy and Phytochemistry, 8(5): 2418–2421.
31. Egbujor, M. C., Ike, S. E., Anieze, E. O., Kanayochukwu, U. L., Nwankwo, N. E., Chidebelu, N. E., Chidebelu, I. C., & Okenwa Ani, C. G. (2019). A comparative study of the phytochemical activities of some Nigerian indigenous kola nuts Kola acuminate, Kola vera, and Garcinia kola. Asian Journal of Applied Chemistry Research, 3(2): 1–6.
32. Soltanian, S., Mohamadi, N., Rajaei, P., Khodami, M., & Mohammadi, M. (2019). Phytochemical composition, and cytotoxic, antioxidant, and antibacterial activity of the essential oil and methanol extract of Semenovia suffruticosa. Avicenna Journal of Phytomedicine, 9: 143–152.
33. Meziti, H., Bouriche, H., Kada, S., Demirtas, I., Kizil, M., & Senator, A. (2019). Phytochemical analysis, and antioxidant, anti-hemolytic and genoprotective effects of Quercus ilex L. and Pinus halepensis Mill. methanolic extracts. Journal of Pharmacy & Pharmacognosy Research, 7(4): 260–272.
34. Ogbiko, C., Achimugu Musa, D., Usman Dabai, M., Jude Ali, I., Sani Yelwa, A., & Buhari Bature, H. (2019). Phytochemical, quantitative proximate and in vitro anti-inflammatory study of the crude methanol extract of Stachytarpheta indica leaves (Verbenaceae). Earthline Journal of Chemical Sciences, 2: 153–162.
35. Yakubul, J., Mamza, U. T., Balami, V. M., Medugu, A. N., Abdulrahman, F. I., & Sodipo, O. A. (2020). Antidiabetic effects of partitioned methanol extract of Boswellia

dalzielii (Frankincense tree) on rats. The Journal of Phytopharmacology, 9(4): 224–229.

36. Dowlath, M. J. H., Karuppannan, S. K., Raiyaan, D. G., Khalith, M. S. B., Subramanian, S., & Arunachalam, K. D. (2020). Effect of solvents on phytochemical composition and antioxidant activity of Cardiospermum halicacabum (L.) extracts. Pharmacognosy Journal, 12(6): 1241–1251.
37. Adusei, S. (2020). Bioactive compounds and antioxidant evaluation of methanolic extract of Hibiscus sabdariffa. The Journal of Technology and Science, 31(2): 0853–4098.
38. Dutta, R., Sharma, M. K., Khan, A., & Jha, M. (2020). Phytochemical and in vitro antioxidant assay of Fumaria officinalis leaf extract. Journal of Advanced Scientific Research, 11(3): 176–182.
39. Tekaday, D., Antony, R., & Jain, S. (2020). Antimicrobial, antioxidant and phytochemical investigation of Thuja occidentalis (Arbor vitae) leave extract. GSC Biological and Pharmaceutical Sciences, 12(03): 108–116.
40. Shefali, S., Rupali, C., Rajan, R., Nitin, S., Anuradha, S., Kamal, D., & Vikram, K. (2021). Effect of solvent on yield, phytochemicals and in-vitro antioxidant potential of Rhododendron arboreum. Research Journal of Pharmacy and Technology, 14: 311–316.
41. Raut, B., Khanal, D. P., & Bhandari, K. (2021). Antioxidant and thrombolytic activities of methanolic extracts of Achyranthes aspera Linn. JMMIHS, 7: 39–48.
42. Chukwube, V. O., Okonta, E. O., Ezugwu, C. O., & Odoh, U. E. (2021). Evaluation of in vitro antioxidant and qualitative phytochemical analysis of methanol leaf extract of Fadogia cienkowskii Scheinf Fam. Rubiaceae. Asian Journal of Research in Medical and Pharmaceutical Sciences, 10(3): 17–23.
43. Shrestha, T., & Lamichhane, J. (2021). Assessment of phytochemicals, antimicrobial, antioxidant and cytotoxicity activity of methanolic extract of Tinospora cordifolia (Gurjo). Nepal Journal of Biotechnology, 9(1): 18–23.
44. Senhaji, S., Lamchori, F., & Toufik, H. (2020). Phytochemical content, antibacterial and antioxidant potential of endemic plant Anabasis aretioïdes Coss. & Moq. (Chenopodiaceae). BioMed Research International, 2020; 01–16.
45. Oryan, A., Hashemnia, M., Hamidi, A. R., & Mohammadalipour, A. (2014). Effects of hydro-ethanol extract of Citrullus colocynthis on blood glucose levels and pathology of organs in alloxan-induced diabetic rats. Asia Pacific Journal of Tropical Disease, 4(2): 125–130.
46. Dowlath, M. J. H., Karuppannan, S. K., Raiyaan, D. G. I., Khalith, M. S. B.,

Subramanian, S., & Arunachalam, K. D. (2020). Effect of solvents on phytochemical composition and antioxidant activity of Cardiospermum halicacabum (L.) extracts. Pharmacognosy Journal, 12(6): 1241–1251.

47. Mac-Kalunta, O. M., Ahamefula, A. A., Odii, C., & Ibe, B. (2022). Comparative studies of the yield and chemical constituents of Bryophyllum pinnatum by cold maceration and sohxlet extraction methods. Journal of Chemical Society of Nigeria, 47: 807–817.
48. Gopalasatheeskumar, K., Kalaichelvan, V. K., Kannappan, N., & Mullai, P. (2022). Different extraction methods for the extraction of phenolics, flavonoids, antioxidant and antidiabetic phytochemicals from Momordica cymbalaria leaves. Indian Journal of Natural Sciences, 12.
49. Yahyaoui, M., Ghazouani, N., Sifaoui, I., & Abderrabba, M. Comparison of the effect of various extraction methods on the phytochemical composition and antioxidant activity of Thymelaea hirsuta L. aerial parts in Tunisia. Biosciences Biotechnology Research Asia, 14(3): 997–1007.
50. Shetty, V., Lobo, R., Kumar, N., Lingadakai, R., Pai, G. C., Balaraju, G., & Ballal, M. (2017). Antimicrobial activity of Anisochilus carnosus (L.f.) Wall against the human gastric pathogen Helicobacter pylori. Asian Journal of Pharmaceutical and Clinical Research, 10: 292–295.
51. George, M., Joseph, L., & Sony, S. (2017). Evaluation of analgesic activity of ethanolic extract of Strobilanthes ciliatus Nees. The Pharma Innovation Journal, 6(7): 326–328.
52. Musdja, M. Y., Mahendra, F., & Musir, A. (2017). Anti-hyperglycemic effect and glucose tolerance of guajava (Psidium guajava L.) leaf ethanol extract in diabetic rats. IOP Conference Series: Earth and Environmental Science.
53. Juluri, K. D. T., Rajan, R. G., & Sara, P. (2018). Biological evaluation of Saussurea lappa root extract for analgesic and anti-inflammatory activity. Asian Journal of Pharmaceutical Research and Development, 6(4): 35–38.
54. Kumar, V., Chakraborty, A., Kaur, M., Pandey, S., & Kuamr Jena, M. (2018). Comparative study on antimicrobial activity of tulsi (Ocimum sanctum) and neem (Azadirachta indica) methanol extract. Asian Journal of Pharmaceutical and Clinical Research, 11: 514–517.
55. Farjadmand, F., Khanavi, M., Eftekhari, M., Hosseinsalari, A., Akbarzadeh, T., Safavi, M., Asatouri, R., Mirabzadeh, M., & Shams Ardekani, M. R. (2017). The effect of extraction method on the major constituents and biological effects of Trachyspermum ammi L. fruits. Research Journal of Pharmacognosy, 5(1): 55–61.

56. Adhikari, P., Pandey, A., Agnihotri, V., & Pande, V. (2018). Selection of solvent and extraction method for determination of antimicrobial potential of Taxus wallichiana Zucc. Research in Pharmacy, 8: 01–09.
57. Matulevich Pelaez, J. A., Castrillón Cardona, W. F., & Torres Torres, K. D. (2018). Phytochemical study of leaves of Muntingia calabura (Muntingiaceae) from Colombia. Indian Journal of Science and Technology, 11(31).
58. Arya, V., Thakur, N., & Kashyap, C. P. (2012). Preliminary phytochemical analysis of the extracts of Psidium leaves. Journal of Pharmacognosy and Phytochemistry, 1.
59. Pundarikakshudu, K., Deepak, H. S., Aashish, H. P., & Gordhanbhai, C. B. (2016). Anti-inflammatory activity of fenugreek (Trigonella foenum-graecum Linn) seed petroleum ether extract. Indian Journal of Pharmacology, 48(4): 441–444.
60. Waled, K. A., Abdulkareem, H. A., Ban, J. Q., & Hayder, B. S. (2016). Antiangiogenesis and antioxidant effect of Anabasis articulata stems extracts. International Journal of Pharmaceutical Sciences Review and Research, 41(2): 88–94.
61. Sahib, H. B., Aisha, A. F., Yam, M. Z., Asmawi, M. Z., Ismail, Z., Salhimi, S. M., Othman, N. H., & Abdul Majid, A. M. S. (2009). Anti-angiogenic and anti oxidant properties of Orthosiphon stamineus Benth. methanolic leaves extract. International Journal of Pharmacology, 5(2): 162–167.
62. Patil, S. P., Kumbhar, S. T., & Ambhore, V. (2017). Evaluation of unsaponified petroleum ether extract of Lantana camara L. leaves for antioxidant activity and oxidative stability. Indian Journal of Pharmaceutical Education and Research, 51(4).
63. Zabihi-nik, T., Hakemi-vala, M., & Baghery-Bejestany, F. (2017). Investigation of antimicrobial effect of crude extract and three sub-fractions of Platychaete aucheri (Boiss.) Boiss against five standard microbial strains and clinical Escherichia coli isolates investigation of antimicrobial effect of crude extract and three sub-fractions of Platychaete aucheri (Boiss.) Boiss against five standard microbial strains and clinical Escherichia coli isolates. Journal of Herbal Drugs, 8: 15–20.
64. Singh, H., Ali, S. S., Khan, N. A., & Mishra, A. K. (2017). Wound healing potential of Cleome viscosa Linn. seeds extract and isolation of active constituent. South African Journal of Botany, 112: 460–465.
65. Srivastava, R., Rurum, A. M., Shehu, P. I. A., & Rajak, C. (2018). Preliminary phytochemical investigation and TLC fingerprint profile of Amaranthus herbs with nutraceutical potential. The Pharma Innovation Journal, 7(5): 224–229.
66. Parveen, S. (2018). Phytochemical screening and antimicrobial activity of herbal plant

extracts- Achyranthus aspera. International Journal of Pharmaceutical and Clinical Research, 10(7): 201–209.

67. Elshiekh, Y. K., & Abdelmageed, M. A. M. (2019). Antioxidant, cytotoxicity and antitumor of Bergia suffruticosa (whole plant). International Journal of Scientific Research in Biological Sciences, 6: 52–55.
68. Friday, A., Abalaka, E. M., Shadrach, A. U., & Tayo, O. T. (2021). In-vitro investigation on the therapeutic potential of Carica papaya leaf extract on some pathogenic bacteria. South Asian Research Journal of Natural Products, 4(1): 9–15.
69. Munnangi, V. (2022). Phytochemical and antioxidant study of Phyllanthus debilis Klein ex Wild. International Journal of Advanced Research in Medical & Pharmaceutical Sciences, 7. ISSN 2455-6998
70. Fissehatsion, S., Girmai, B., Berhane, B., & Berhane, S. (2022). Anti-microbial and phytochemical screening of Commiphora africana, Cucumis pustulatus and Vernonia schimperi from Eritrea. Research Square.
71. Agboke, A. A., & Attama, A. A. (2016). Bioactive components and antibacterial activities of n-hexane extract of Moringa oleifera root bark on clinical isolates of methicillin resistant Staphylococcus aureus. International Journal of Current Research in Chemistry and Pharmaceutical Sciences, 3(3): 1–9.
72. Joshua, P. E., Oka, S. A., & Eze, C. S. (2017). Estimation of phytochemical, total phenolic and total flavonoid contents of methanol extract of Voacanga africana root bark and its fractions. Bio-Research, 15: 988–993.
73. Yahyaoui, M., Chazouani, N., Sifaoui, I., & Abderrabba, M. (2017). Comparison of the effect of various extraction methods on the phytochemical composition and antioxidant activity of Thymelaea hirsuta L. aerial parts in Tunisia. Biosciences Biotechnology Research Asia, 14(3): 997–1007.
74. Gololo, S. S., Mapfumari, N. S., Sethoga, L. S., Olivier, M. T., Shai, L. J., & Mogale, M. A. (2016). Identification of phytochemical constituents within the n-hexane leaf extract of Senna italica (Mill) using gas chromatography-mass spectrometry (GC-MS) analysis. Journal of Pharmaceutical Sciences & Research, 8(10): 1141–1143.
75. Francis, G. A., Olalekan, O. A., Ogochukwu, U. K., & Eshiokhede, A. (2016). Active phytochemicals and antimicrobial properties of the extracts of Xylopia aethiopica root and stem bark. Journal of Complementary and Alternative Medical Research, 1(2): 1–5.
76. Umar, H., Umar, I. A., & Ibrahim, A. (2017). Phytochemical analysis and in-vitro anti plasmodia activity of Chrozophora senegalensis extracts on Plasmodium falciparum.

Nigerian Journal of Chemical Research, 22.

77. Lubna, T., Aslam, A., & Ahmed, S. (2017). Antibacterial activities of Diospyros blancoi, Phoenix dactylifera and Morus nigra against dental caries causing pathogens: An in vitro study. Pakistan Journal of Pharmaceutical Sciences, 30: 163–169.
78. Reshman, A. U., Latif, A., Abbas, N., Waheed, I., Reshman, A. U., & Qaisar, M. N. (2019). Alpha-glucosidase inhibitory and antioxidant activities of various extracts of aerial parts of Fagonia indica Burm. F. Tropical Journal of Pharmaceutical Research, 18(4): 791–797.
79. Muhammad, A. A., Suleman, L., & Salisu, A. J. (2019). Phytochemical screening and elemental analysis of crude extract of Mentha piperita (Peppermint) leaves. Chemistry Research Journal, 4(4): 35–40.
80. Hanani, E., Soewandi, S. W., Hayati, & Revita, N. (2019). Pharmacognostical and preliminary phytochemical evaluation of Cordia sebestena L. Pharmacognosy Journal, 11(5): 110–1105.
81. Zemene, M., Geta, M., Huluka, S. A., & Birru, E. M. (2020). Antimalarial activity of the 80% methanol leaf extract and solvent fractions of Stephania abyssinica (Dill. & A. Rich.) Walp. against Plasmodium berghei infection in mice. Ethiopian Pharmaceutical Journal, 36: 109–120.