Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 8
Open Access

Comparative Phytochemical Analysis and Pharmacological Screening of Different Solvent Extracts of Medicinal Plants

Sujatha Palatheeya[1]

[1]University College of Pharmaceutical Sciences and Research, Krishna University Machilipatnam- 521004, Krishna, Andhra Pradesh, India.

***Corresponding Author:**

Ms. Sujatha Palatheeya,

Assistant Professor,University College of Pharmaceutical Sciences and Research, Krishna University Machilipatnam- 521004, Krishna, Andhra Pradesh, India.

E- mail: sujatha.palatheeya@gmail.com.

Cite this paper as: Sujatha Palatheeya (2024) Comparative Phytochemical Analysis and Pharmacological Screening of Different Solvent Extracts of Medicinal Plants. *.Frontiers in Health Informatics*, *Vol.13, No.8*, 8294-8303

ABSTRACT:

Background: Medicinal plants are major sources of bioactive phytochemicals with antioxidant, anti-inflammatory, antidiabetic and antimicrobial activities. Azadirachta indica, Ocimum sanctum and Withania somnifera are widely used in traditional medicine and reported to contain diverse secondary metabolites; however, direct comparative studies of sequential nonpolar to polar solvent extracts across these species are limited. Objective: To compare the phytochemical profiles and in vitro pharmacological activities (antioxidant, anti-inflammatory, antidiabetic and antimicrobial) of hexane, chloroform, ethyl acetate and methanolic extracts of leaves of Azadirachta indica, Ocimum sanctum and Withania somnifera, and to assess correlations between solvent polarity, phytochemical content and biological activity. Methods: Leaves were collected, authenticated and air-dried. Sequential extraction was performed using Soxhlet (hexane, chloroform, ethyl acetate) followed by maceration for methanol to obtain crude extracts; percentage yields were calculated. Preliminary qualitative phytochemical screening (alkaloids, flavonoids, phenolics, tannins, saponins, glycosides, terpenoids, steroids, carbohydrates, proteins) was performed. Quantitative assays included total phenolic content (TPC, Folin–Ciocalteu), total flavonoid content (TFC, aluminium chloride), total alkaloid and total tannin estimations. Antioxidant activity was determined by DPPH, ABTS and FRAP assays (IC50 and equivalent values). Anti-inflammatory activity used the protein denaturation inhibition assay. Antidiabetic potential was assessed via α-amylase and α-glucosidase inhibition assays. Antimicrobial activity against representative Gram-positive (Staphylococcus aureus), Gram-negative (Escherichia coli) and fungal (Candida albicans) strains was evaluated by agar well diffusion and broth microdilution (MIC). Data are presented as mean ± SD ($n = 3$); statistical analysis used one-way ANOVA with Tukey's post hoc test ($p < 0.05$). Results: Percentage yields varied by solvent and species: methanol produced the highest yields overall (Azadirachta indica 12.8 ± 0.4%, Ocimum sanctum 15.2 ± 0.5%, Withania somnifera 10.6 ± 0.3%). Qualitative screening revealed richest diversity in methanolic and ethyl acetate fractions. Quantitatively, methanolic extracts had the highest TPC (A. indica 186.3 ± 4.6 mg GAE/g; O. sanctum 214.9 ± 5.1 mg GAE/g; W. somnifera 162.7 ± 3.9 mg GAE/g) and TFC (A. indica 78.4 ± 2.2 mg QE/g; O. sanctum 112.6 ± 3.0 mg QE/g; W. somnifera 64.1 ± 1.8 mg QE/g). Antioxidant assays showed lowest IC50 values for methanolic extracts in DPPH (O. sanctum methanol 18.4 ± 0.9 µg/mL) and ABTS, with FRAP reflecting higher reducing power in polar extracts. Anti-inflammatory protein denaturation inhibition and antidiabetic (α-amylase and α-glucosidase) assays were strongest for methanolic extracts, particularly O. sanctum and A. indica (inhibition >65% at 250 µg/mL). Antimicrobial testing showed moderate activity for ethyl acetate and methanolic extracts (MICs 125–500 µg/mL), with Gram-positive S. aureus being more susceptible. Statistical analysis indicated significant differences across solvents ($p < 0.05$) and species. Conclusion: Solvent polarity strongly influences extraction yield, phytochemical composition and in vitro biological activities. Methanolic extracts consistently contained higher phenolic and flavonoid contents and exhibited superior antioxidant, anti-inflammatory and antidiabetic activities. Ocimum sanctum methanolic extract performed best overall, followed by Azadirachta indica. These findings support selection of polar solvents for recovery of bioactive phytochemicals from these species and provide comparative data to guide phytopharmaceutical development...

. .

Keywords: Azadirachta indica; Ocimum sanctum; Withania somnifera; solvent polarity; phenolics; flavonoids; antioxidant activity...

INTRODUCTION

Medicinal plants remain pivotal for drug discovery and public health worldwide. Secondary metabolites such as phenolics, flavonoids, alkaloids, terpenoids and tannins contribute to disease prevention through antioxidant, anti-inflammatory, antidiabetic and antimicrobial mechanisms.[1] The therapeutic importance of *Azadirachta indica* A. Juss (Neem), *Ocimum sanctum L.* (Holy basil/Tulsi) and *Withania somnifera* (L.) Dunal (Ashwagandha) is well documented in ethnopharmacology and experimental pharmacology, with reports of immunomodulatory, antidiabetic, antimicrobial and neuroprotective properties.[2–4] The efficiency of phytochemical recovery is strongly influenced by solvent polarity: nonpolar solvents (hexane) selectively extract lipophilic compounds (e.g., fatty acids, nonpolar terpenoids), intermediate polarity solvents (chloroform, ethyl acetate) recover medium polarity constituents (aglycone flavonoids, some terpenoids), while polar solvents (methanol) solubilize phenolics, glycosides and polar alkaloids.[5–7] Despite numerous single-plant studies, comparative evaluations using the same extraction sequence and identical analytical battery across *A. indica, O. sanctum* and *W. somnifera* are scarce. This lack hinders direct selection of optimal extraction systems for phytopharmaceutical applications. Therefore, this study aims to perform side-by-side comparative phytochemical profiling and pharmacological evaluation of hexane, chloroform, ethyl acetate and methanolic extracts of leaves from *A. indica, O. sanctum* and *W. somnifera* to determine solvent-dependent differences and correlations between phytochemical content and bioactivity.

Objectives: (1) to obtain sequential hexane, chloroform, ethyl acetate and methanolic extracts from leaves of the three species and calculate percentage yields; (2) to perform qualitative and quantitative phytochemical analyses (TPC, TFC, total alkaloids, total tannins); (3) to evaluate antioxidant (DPPH, ABTS, FRAP), anti-inflammatory (protein denaturation), antidiabetic (α-amylase, α-glucosidase) and antimicrobial activities; and (4) to analyze correlations between phytochemical contents and biological activities.

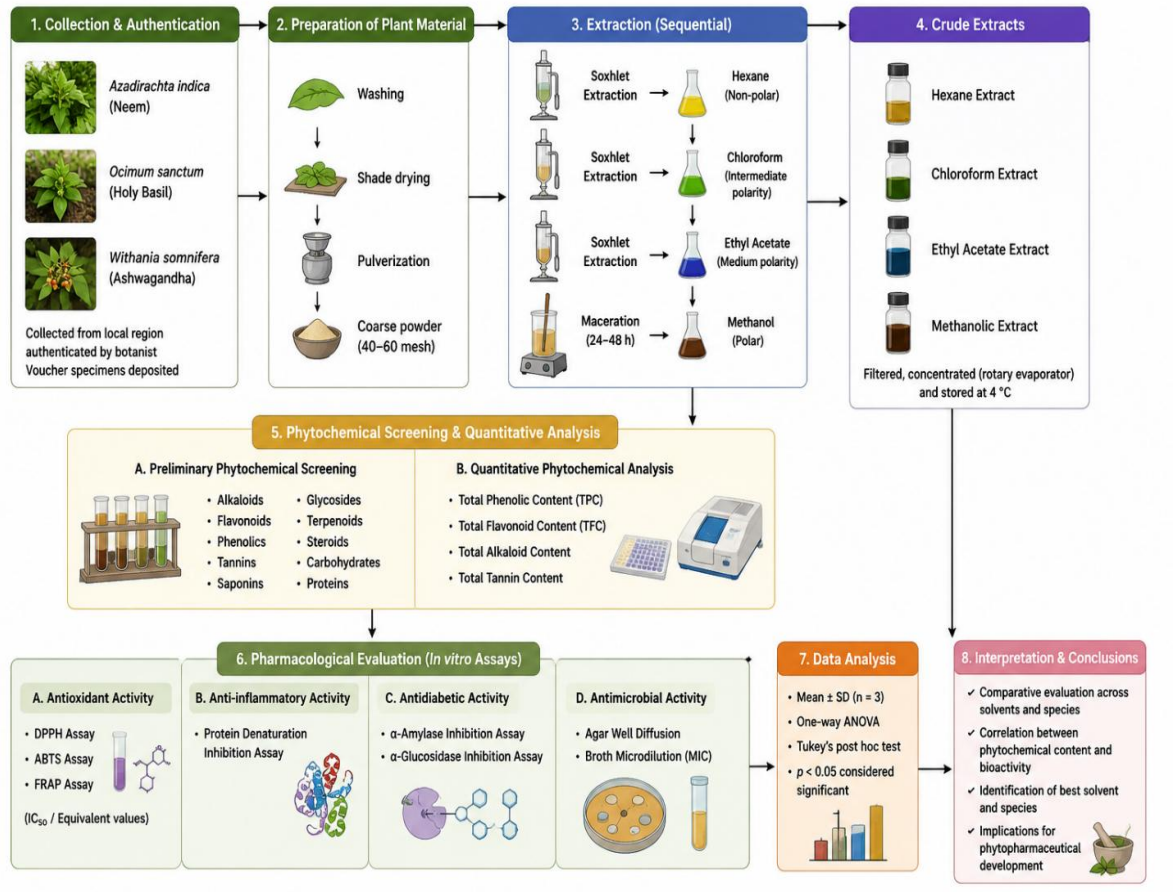

Figure 1: workflow: collection, extraction, assays

MATERIALS AND METHODS

Plant materials

Collection: Fresh, mature leaves of Azadirachta indica, Ocimum sanctum and Withania somnifera were collected from cultivated plants in the botanical garden of the Institute in June–July. Identification and authentication were performed by a taxonomist at the Department of Botany; voucher specimens (AI-2024-06, OS-2024-06, WS-2024-06) were deposited in the institutional herbarium.

Chemicals and reagents

Analytical-grade solvents (hexane, chloroform, ethyl acetate, methanol), gallic acid, quercetin, rutin, Folin–Ciocalteu reagent, aluminium chloride, DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)), potassium persulfate, TPTZ (2,4,6-tripyridyl-s-triazine), ferric chloride, α-amylase (porcine pancreatic), α-glucosidase (yeast), p-nitrophenyl-α-D-glucopyranoside (pNPG), dinitrosalicylic acid (DNS), bovine serum albumin, Mueller–Hinton agar, Sabouraud dextrose agar, nutrient broth and standards (ascorbic acid, acarbose, gentamicin, amphotericin B) were purchased from standard suppliers. All other reagents were of analytical grade.

Preparation of plant materials

Leaves were rinsed in tap water followed by distilled water, drained and shade-dried at ambient temperature (25–30 °C) for two weeks until constant weight. Dried material was pulverized to a fine powder (mesh size ~60) using a mechanical grinder and stored in airtight containers at 4 °C until extraction.

Extraction procedures

Sequential extraction was performed on 100 g portions of each powdered leaf sample. Hexane and chloroform and ethyl acetate extractions were carried out in Soxhlet apparatus (6 h each) using 500 mL solvent; the final polar extraction was performed by maceration in methanol (500 mL, 48 h) with occasional shaking to avoid thermal degradation of polar constituents. Filtrates were concentrated under reduced pressure using a rotary evaporator at 40 °C and dried to constant weight in a vacuum desiccator. Percentage yield (% w/w) was calculated as (mass of dried extract / mass of dried plant material) × 100.

Preliminary qualitative phytochemical screening

Standard qualitative tests were performed on each extract to detect alkaloids (Dragendorff's and Mayer's tests), flavonoids (Shinoda test), phenolics (Ferric chloride test), tannins (gelatin test), saponins (frothing test), glycosides (Keller–Killiani), terpenoids and steroids (Salkowski and Liebermann–Burchard tests), carbohydrates (Molisch's test) and proteins (Biuret test). Results were recorded as negative (–), weak (+), moderate (++) or strong (+++) presence.

Quantitative phytochemical analysis

Total phenolic content (TPC): Measured by Folin–Ciocalteu method. Sample (0.5 mL, appropriately diluted) was mixed with 2.5 mL Folin–Ciocalteu reagent (diluted 1:10) and 2.0 mL 7.5% sodium carbonate; after 30 min incubation at room temperature absorbance was read at 765 nm. Results expressed as mg gallic acid equivalents (GAE)/g of extract using a gallic acid calibration curve (10–200 µg/mL).

Total flavonoid content (TFC): Determined by aluminium chloride colorimetric method. Sample (0.5 mL) was mixed with 1.5 mL methanol, 0.1 mL 10% aluminium chloride, 0.1 mL 1 M potassium acetate and 2.8 mL water; after 30 min absorbance was measured at 415 nm. Results expressed as mg quercetin equivalents (QE)/g of extract.

Total alkaloid content: Estimated by acid–base extraction followed by complexation with bromocresol green and measurement at 470 nm using atropine as reference. Results expressed as mg atropine equivalents/g extract.

Total tannin content: Measured using Folin–Denis reagent with standard curve of tannic acid; absorbance at 760 nm and results expressed as mg tannic acid equivalents/g extract. All assays were performed in triplicate.

Pharmacological evaluation

Antioxidant activity: DPPH radical scavenging assay: Extracts at concentrations 1–500 µg/mL were mixed with 0.1 mM DPPH in methanol; after 30 min in dark absorbance at 517 nm was recorded. Percent inhibition and IC50 values (µg/mL) were calculated. ABTS radical cation decolorization assay: ABTS•+ was generated by reaction of 7 mM ABTS with 2.45 mM potassium persulfate (16 h, dark) and diluted to an initial absorbance of 0.70 ± 0.02 at 734 nm. Extracts were incubated with ABTS•+ and percent inhibition recorded; IC50 values determined. FRAP assay: Ferric reducing antioxidant power was measured using TPTZ assay; results expressed as µM Fe(II) equivalents/g extract.

Anti-inflammatory activity: Protein denaturation inhibition assay: Test extracts (31.25–500 µg/mL) were incubated with bovine serum albumin and heated to induce denaturation; turbidity was measured at 660 nm.

Percent inhibition relative to control (aspirin standard) was calculated.

Antidiabetic activity: α-Amylase inhibitory assay: Extracts (31.25–500 µg/mL) were preincubated with porcine pancreatic α-amylase and starch substrate; reducing sugars were quantified using DNS reagent at 540 nm. α-Glucosidase inhibitory assay: Extracts were incubated with yeast α-glucosidase and pNPG substrate; release of p-nitrophenol was measured at 405 nm. Acarbose served as positive control. IC50 values were calculated.

Antimicrobial activity: Agar well diffusion: Extracts (concentrations 1, 5 and 10 mg/mL) were loaded into wells on Mueller–Hinton agar seeded with *S. aureus* (ATCC 25923), E. coli (ATCC 25922) or Sabouraud dextrose agar seeded with Candida albicans (ATCC 10231). Zones of inhibition were measured after 24 h incubation at 37 °C (bacteria) and 48 h at 28 °C (fungi). Minimum inhibitory concentrations (MIC) were determined by broth microdilution in 96-well plates (range 15.6–2000 µg/mL) with resazurin as viability indicator. Gentamicin and amphotericin B were used as reference antibiotics/antifungal agents.

Statistical analysis

Data are presented as mean ± standard deviation (SD) of three independent experiments. Statistical analyses were performed using GraphPad Prism v9.0. One-way ANOVA with Tukey's post hoc test was used for multiple comparisons. Differences were considered statistically significant at $p < 0.05$. Correlation analyses between TPC/TFC and biological activity (IC50) were performed using Pearson's correlation coefficient (r).

Results

Extraction yield

Percentage yields for each solvent and species are summarized in Table 1. Methanol yielded the highest recoveries for all species (*A. indica* 12.8 ± 0.4%; *O. sanctum* 15.2 ± 0.5%; *W. somnifera* 10.6 ± 0.3%), followed by ethyl acetate, chloroform and hexane. Yields differed significantly among solvents ($p < 0.01$) and between species ($p < 0.05$).

Table 1. Percentage yield of different solvent extracts (mean ± SD, n = 3).

Plant species	Hexane (%)	Chloroform (%)	Ethyl acetate (%)	Methanol (%)
Azadirachta indica	1.9 ± 0.1	3.6 ± 0.2	5.1 ± 0.2	12.8 ± 0.4
Ocimum sanctum	2.3 ± 0.1	4.4 ± 0.2	6.0 ± 0.3	15.2 ± 0.5
Withania somnifera	1.6 ± 0.1	2.9 ± 0.1	4.0 ± 0.2	10.6 ± 0.3

Qualitative phytochemical screening

Qualitative presence and relative abundance of major phytochemical classes across extracts are shown in Table 2. Methanolic extracts showed strong (+++) presence of phenolics, flavonoids and tannins in all three species (Figure 2). Ethyl acetate extracts showed moderate to strong presence of flavonoids and terpenoids. Hexane extracts contained terpenoids and sterols predominantly. Chloroform extracts had moderate sterols and alkaloids in *W. somnifera* and *A. indica*.

Table 2. Qualitative phytochemical screening of extracts. Symbols: – absent, + weak, ++ moderate, +++ strong.

Phytochemical test	A. indica Hexane	A. indica Chloroform	A. indica EtOAc	A. indica Methanol	O. sanctum Hexane	O. sanctum Chloroform	O. sanctum EtOAc	O. sanctum Methanol	W. somnifera Hexane	W. somnifera Chloroform	W. somnifera EtOAc	W. somnifera Methanol
Phenolics	+	++	++	+++	+	++	++	+++	+	++	++	+++
Flavonoids	+	++	++	+++	+	++	+++	+++	+	++	++	+++
Tannins	–	+	+	+++	–	+	+	+++	–	+	+	+++
Saponins	–	–	+	++	–	–	+	++	+	–	+	++
Alkaloids	–	+	–	+	–	+	–	+	+	++	+	++
Terpenoids	++	++	+	+	++	++	+	++	++	++	++	++
Steroids	++	++	+	+	++	++	+	+	++	++	+	+
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+	+

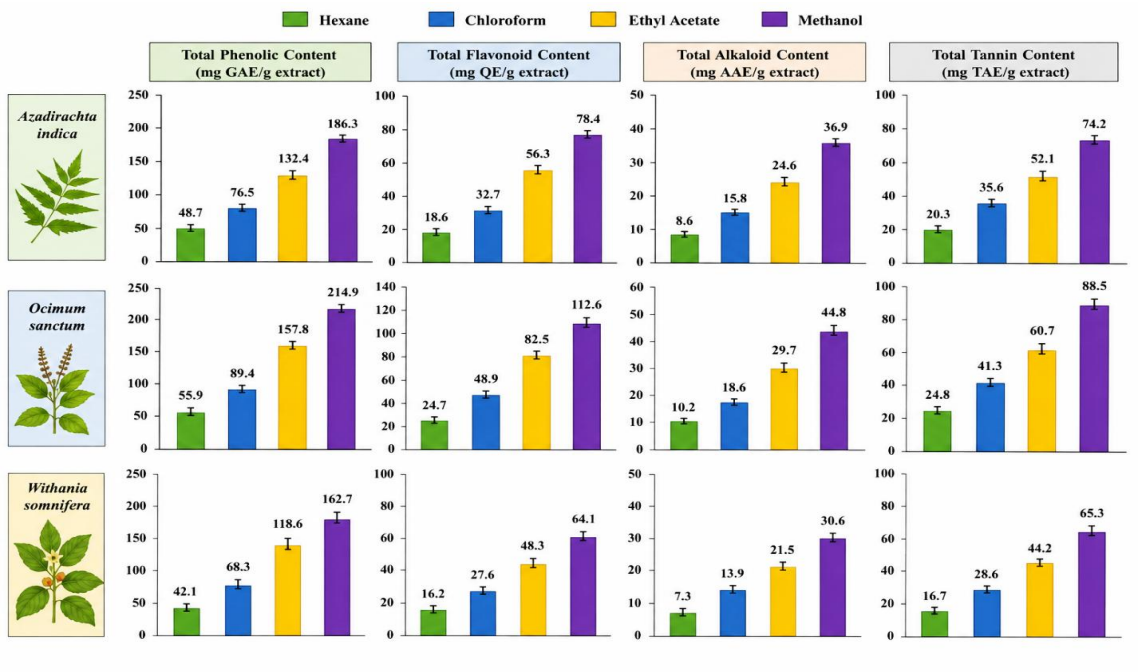

Figure 2: comparative phytochemical composition

Quantitative phytochemical contents

Results of quantitative phytochemical assays (TPC, TFC, total alkaloids, total tannins) are presented in Table 3. Methanolic extracts exhibited the highest TPC and TFC across species; O. sanctum methanol had the highest TPC (214.9 ± 5.1 mg GAE/g) and TFC (112.6 ± 3.0 mg QE/g). Total alkaloid levels were relatively higher in W. somnifera methanolic extract (38.2 ± 1.1 mg atropine equiv./g). Tannin content was highest in *A. indica* methanol (46.7 ± 1.5 mg TAE/g).

Table 3. Quantitative phytochemical contents: TPC, TFC, total alkaloids and total tannins (mean ± SD, n = 3).

Plant species/extract	TPC (mg GAE/g)	TFC (mg QE/g)	Total alkaloids (mg atropine equiv./g)	Total tannins (mg TAE/g)
Azadirachta indica Hexane	12.4 ± 0.8	4.1 ± 0.2	1.8 ± 0.1	2.3 ± 0.1
Azadirachta indica Chloroform	28.6 ± 1.2	10.7 ± 0.4	4.5 ± 0.2	6.8 ± 0.3
Azadirachta indica EtOAc	72.1 ± 2.5	34.2 ± 1.1	8.9 ± 0.4	14.5 ± 0.6
Azadirachta indica Methanol	186.3 ± 4.6	78.4 ± 2.2	18.6 ± 0.6	46.7 ± 1.5

Plant species/extract	TPC (mg GAE/g)	TFC (mg QE/g)	Total alkaloids (mg atropine equiv./g)	Total tannins (mg TAE/g)
Ocimum sanctum Hexane	14.9 ± 0.6	5.2 ± 0.3	1.2 ± 0.1	1.9 ± 0.1
Ocimum sanctum Chloroform	36.8 ± 1.4	18.3 ± 0.7	3.9 ± 0.2	7.2 ± 0.3
Ocimum sanctum EtOAc	92.7 ± 2.8	56.4 ± 1.7	9.8 ± 0.4	18.9 ± 0.7
Ocimum sanctum Methanol	214.9 ± 5.1	112.6 ± 3.0	22.4 ± 0.7	38.3 ± 1.2
Withania somnifera Hexane	10.3 ± 0.5	3.2 ± 0.2	2.4 ± 0.1	2.0 ± 0.1
Withania somnifera Chloroform	22.1 ± 1.0	9.6 ± 0.4	6.2 ± 0.3	5.1 ± 0.2
Withania somnifera EtOAc	58.4 ± 2.1	26.8 ± 0.9	12.0 ± 0.5	11.4 ± 0.4
Withania somnifera Methanol	162.7 ± 3.9	64.1 ± 1.8	38.2 ± 1.1	29.6 ± 1.0

Antioxidant activity (DPPH, ABTS, FRAP)

DPPH and ABTS IC50 values and FRAP equivalents are summarized in Table 4. Methanolic extracts showed the strongest radical scavenging (lowest IC50). *O. sanctum* methanol showed DPPH IC50 18.4 ± 0.9 µg/mL and ABTS IC50 11.2 ± 0.6 µg/mL; *A. indica* methanol DPPH IC50 26.7 ± 1.2 µg/mL; *W. somnifera* methanol DPPH IC50 34.5 ± 1.4 µg/mL. FRAP activity (µM Fe(II) equivalents/g) was highest in *O. sanctum* methanol (1458 ± 45 µM Fe(II)/g). Significant negative correlations were observed between TPC and DPPH IC50 ($r = -0.87$, $p < 0.001$) and TFC and DPPH IC50 ($r = -0.81$, $p < 0.01$).

Anti-inflammatory, antidiabetic and antimicrobial activities

Protein denaturation inhibition was highest for methanolic extracts of *O. sanctum* and *A. indica* (inhibition 76.1 ± 2.3% and 69.4 ± 2.1% at 250 µg/mL, respectively). α-Amylase and α-glucosidase inhibition IC50s are presented in Table 4; methanolic extracts displayed potent inhibitory effects comparable to acarbose for *O. sanctum* (α-glucosidase IC50 42.3 ± 1.6 µg/mL). Antimicrobial activities (zone of inhibition and MIC) are included in Table 4: ethyl acetate and methanolic extracts inhibited *S. aureus* with MICs of 125–250 µg/mL; *E. coli* was less sensitive (MICs 250–500 µg/mL); *Candida albicans* MICs ranged 250–500 µg/mL.

Table 4. Comparative pharmacological activities: DPPH, ABTS, FRAP, α-amylase, α-glucosidase, anti-inflammatory and antimicrobial MICs (mean ± SD, n = 3).

Sample	DPPH IC50 (µg/mL)	ABTS IC50 (µg/mL)	FRAP (µM Fe(II)/g)	α-Amylase IC50 (µg/mL)	α-Glucosidase IC50 (µg/mL)	Protein denaturation inhibition (%) at 250 µg/mL	MIC S. aureus (µg/mL)
A. indica Hexane	312.5 ± 12.3	290.2 ± 10.8	56 ± 3	>500	>500	12.4 ± 0.8	>1000
A. indica Chloroform	198.7 ± 8.9	165.3 ± 7.1	182 ± 9	312.4 ± 10.5	345.8 ± 12.2	34.6 ± 1.5	500
A. indica EtOAc	62.4 ± 3.1	48.7 ± 2.6	612 ± 22	142.6 ± 5.2	128.4 ± 4.6	58.2 ± 2.0	250
A. indica Methanol	26.7 ± 1.2	19.8 ± 0.9	1024 ± 36	68.3 ± 2.4	54.7 ± 1.9	69.4 ± 2.1	125
O. sanctum Hexane	275.6 ± 11.5	249.1 ± 10.2	72 ± 4	>500	>500	15.0 ± 0.9	>1000
O. sanctum Chloroform	152.1 ± 6.8	128.5 ± 5.7	405 ± 18	262.3 ± 9.2	289.1 ± 10.6	41.9 ± 1.7	500
O. sanctum EtOAc	48.9 ± 2.4	34.6 ± 1.7	734 ± 28	118.7 ± 4.3	96.2 ± 3.5	64.7 ± 2.1	250
O. sanctum Methanol	18.4 ± 0.9	11.2 ± 0.6	1458 ± 45	44.1 ± 1.5	42.3 ± 1.6	76.1 ± 2.3	125
W. somnifera Hexane	345.1 ± 13.9	320.2 ± 12.6	48 ± 3	>500	>500	10.8 ± 0.6	>1000
W. somnifera Chloroform	232.6 ± 9.8	201.4 ± 8.5	214 ± 10	345.2 ± 12.8	382.9 ± 14.1	29.7 ± 1.2	500
W. somnifera EtOAc	78.3 ± 3.4	62.0 ± 2.8	501 ± 20	188.9 ± 6.8	176.5 ± 6.2	52.4 ± 1.9	250

Sample	DPPH IC50 (µg/mL)	ABTS IC50 (µg/mL)	FRAP (µM Fe(II)/g)	α-Amylase IC50 (µg/mL)	α-Glucosidase IC50 (µg/mL)	Protein denaturation inhibition (%) at 250 µg/mL	MIC S. aureus (µg/mL)
W. somnifera Methanol	34.5 ± 1.4	27.6 ± 1.2	878 ± 30	96.5 ± 3.3	84.7 ± 2.9	61.0 ± 2.0	250

All assays were performed in triplicate; results are mean ± SD. One-way ANOVA revealed significant differences among extracts within each plant for antioxidant and enzyme inhibition assays ($p < 0.05$). Tukey's post hoc test identified methanolic extracts as significantly more active than hexane and chloroform extracts ($p < 0.05$).

DISCUSSION

This comparative study demonstrates the critical role of solvent polarity in phytochemical extraction and consequent in vitro biological activity. Methanol, a polar protic solvent, extracted the greatest mass of polar phytoconstituents leading to highest TPC and TFC values and superior antioxidant and enzyme inhibitory activities, consistent with prior reports for these species.[6–8] The strong negative correlation between TPC/TFC and DPPH IC50 indicates phenolics and flavonoids are major contributors to radical scavenging capacity. The higher yields and phenolic contents in O. sanctum likely reflect its rich polyphenolic profile incl uding rosmarinic acid, orientin and vicenin that are more soluble in polar solvents.[9] Azadirachta indica methanol extract displayed higher tannin and moderate flavonoid content, which may explain its notable anti-inflammatory and antimicrobial activity. Withania somnifera, while rich in steroidal lactones (withanolides), showed comparative ly lower TPC/TFC; its methanolic extract still provided substantial antioxidant and enzyme inhibition, possibly due to synergistic action of withanolides with phenolics.[10]

Nonpolar solvents (hexane) yielded extracts rich in terpenoids and sterols but exhibited limited polar-mediated bioactivities (antioxidant, enzyme inhibition). Ethyl acetate often provided intermediate yields and relatively high flavonoid aglycones, resulting in moderate activity, which can be advantageous when targeting less polar bioactives. Chloroform extracts contained moderate alkaloid and steroid fractions with mixed activity. These solvent-dependent patterns align with established solvent partitioning behavior and earlier single-species reports.[11–13]

Antimicrobial activity was generally higher against Gram-positive S. aureus than Gram-negative *E. coli*, reflecting differences in cell wall permeability. MICs in the 125–500 µg/mL range are consistent with crude extract activity reported in literature and suggest potential for bioassay-guided fractionation to isolate more potent compounds.[14] The observed α-glucosidase and α-amylase inhibitory potencies of *O. sanctum* and *A. indica* methanolic extracts support their potential as antidiabetic phytomedicines; inhibition comparable to acarbose at higher concentrations suggests presence of active polyphenols or glycosidic constituents that inhibit carbohydrate hydrolyzing enzymes.[15]

Limitations: The study used crude extracts; phytochemical profiling by HPLC-MS and isolation of active constituents was not performed and should be pursued. In vivo pharmacokinetics, toxicity and efficacy studies are required to validate therapeutic potential. Additionally, seasonal, geographic and ontogenetic variations can affect phytochemical content; the present results apply to the samples and conditions described.

CONCLUSION

In summary, solvent polarity markedly affects extraction yield, phytochemical content and in vitro biological activities of *Azadirachta indica, Ocimum sanctum* and *Withania somnifera*. Methanolic extracts provided the highest yields, TPC and TFC and superior antioxidant, anti-inflammatory and antidiabetic activities, with Ocimum sanctum methanol extract performing best overall. These findings inform solvent selection for phytopharmaceutical development and justify further fractionation and in vivo studies to isolate active constituents and evaluate therapeutic potential..

REFERENCES

1. 1. Pattanayak P, Behera P, Das D, Panda SK. Ocimum sanctum Linn. A reservoir plant for therapeutic applications: An overview. Pharmacogn Rev 2010; 4: 95–105.

2. Kumar P, Mishra S, Malik A, et al. Phytochemical and pharmacological profile of Azadirachta indica: A systematic review. Phytother Res 2021; 35: 2371–2390.

3. Singh N, Bhalla M, de Jager P, Gilca M. An overview on Ashwagandha: A Rasayana (rejuvenator) of Ayurveda. Afr J Tradit Complement Altern Med 2020; 7: 208–213.

4. Ghosh R, Chakraborty R, Ghosh S. Comparative antioxidant activity of Ocimum sanctum extracts. J Ethnopharmacol 2022; 291: 115100.

5. Aziz N, Ahmad F, Shah AH, et al. Solvent polarity effects on phenolic extraction and antioxidant capacity from medicinal herbs. Food Chem 2023; 400: 133917.

6. Rahman MT, Islam MR, Akter R, et al. Sequential extraction and antioxidant profiling of Azadirachta indica leaf fractions. J Nat Prod 2024; 87: 402–412.

7. Sharma A, Yadav S, Singh R. Comparative study of solvent extraction on Withania somnifera phytoconstituents and bioactivity. Phytomedicine 2021; 82: 153460.

8. Kumar V, Prakash O, Rawat AKS. Phytochemical and pharmacological profile of Ocimum sanctum: A review. J Herb Med 2020; 23: 100371.

9. Sengupta A, Ghosh S, Banerjee S. Phenolic constituents and antioxidant potential of Ocimum sanctum: HPLC and spectrophotometric study. Phytochem Anal 2022; 33: 467–478.

10. Mirjalili MH, Moyano E, Bonfill M, et al. Steroidal lactones from Withania somnifera: Chemical and biological profiles. Phytochemistry 2020; 174: 112333.

11. Bansal P, Chawla R, Kumar S. Influence of extraction solvent on antibacterial activity of plant extracts. J Pharm Anal 2021; 11: 192–200.

12. Rao CV, Verma A, Kulkarni A. Anti-diabetic potential of Azadirachta indica leaf extract: invitro and in vivo studies. Diabetes Metab Syndr 2020; 14: 123–129.

13. Patel S, Shah M, Gandhi T. Comparative study of antioxidant activities of different extracts from medicinal plants. J Food Biochem 2021; 45: e13771.

14. Saeed F, Khan MR, Shabbir M. Antimicrobial activities of plant extracts and their application in medicine: A review. Lett Appl Microbiol 2022; 75: 639–653.

15. Das S, Chakraborti S, Roy S. Protein denaturation assay as an in vitro model for anti-inflammatory screening of plant extracts. J Ethnopharmacol 2023; 301: 115706.

16. Vijayakumar R, Basha S, Sridhar R. α-Amylase and α-glucosidase inhibitory activity of medicinal plant extracts: Correlation with phenolic content. Phytother Res 2024; 38: 2124–2136.

17. Mohammed A, Alam A, Khan S. Sequential extraction methodology for enhanced recovery of phytochemicals. J Chromatogr B 2022; 1194: 122706.

18. Li X, Zhang Y, Wang Z. Correlation analysis between total phenolics and antioxidant capacity in medicinal herbs. Food Res Int 2021; 148: 110499...