

Comparative Study of Phytochemicals in Selected Indigenous Plant Species of Wardha Region, Maharashtra, India

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Abstract: Indigenous plants represent valuable sources of bioactive secondary metabolites with potential nutritional, medicinal, and pharmacological significance. The present study aimed to comparatively evaluate the qualitative phytochemical composition of total phenolic content (TPC) and total flavonoid content (TFC) of five indigenous plant species from the Wardha region of Maharashtra, India. Leaves of *Aegle marmelos* (L.) Corrêa, *Azadirachta indica* A. Juss., *Achyranthes aspera* L., *Senna tora* (L.) Roxb., and *Clerodendrum infortunatum* L. were collected, shade-dried, powdered, and extracted using 70% ethanol at a plant material-to-solvent ratio of 1:10 (w/v). Preliminary qualitative phytochemical screening was performed to detect alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, steroids, and glycosides. Total phenolic and flavonoid contents were estimated using the Folin–Ciocalteu and aluminium chloride colorimetric methods, respectively, with results expressed as mean $\pm$ standard deviation from triplicate measurements. Flavonoids, phenolic compounds, tannins, and saponins were detected in all five species, whereas the occurrence of alkaloids, terpenoids, steroids, and glycosides varied among the extracts. *A. marmelos* and *S. tora* exhibited the broadest qualitative phytochemical profiles, with all eight tested groups detected. The highest TPC and TFC were recorded in *A. indica* at 72.58 ± 1.74 mg GAE/g and 89.46 ± 2.05 mg QE/g dry extract, respectively, followed by *A. aspera* at 68.42 ± 1.56 mg GAE/g and 76.35 ± 1.84 mg QE/g. *C. infortunatum* showed the lowest TPC and TFC values. The findings demonstrate considerable interspecific variation in the qualitative and quantitative phytochemical characteristics of the studied plants. Under the experimental conditions, *A. indica* and *A. aspera* emerged as promising sources of phenolic and flavonoid constituents for further phytochemical and biological investigations. However, the results represent preliminary screening and should not be interpreted as confirmation of individual compounds or biological activity. It was found that phytochemical composition varied across species studied in terms of detected metabolites and their contents. The results might be used for further phytochemical screening of indigenous plant species occurring in the Wardha region.

Keywords: Indigenous plants, phytochemical screening, phenolic content, flavonoid content, etc.

I. INTRODUCTION

Plants have traditionally been used to satisfy the food, medicinal, fiber, dye and other societal needs. India is endowed with wealth of plant species including the number of indigenous plants used by the rural and tribal population for different purposes. Such information is critical in identifying plants with potential nutritional and medical properties. The biological and medicinal actions are mainly caused by the wide variety of phytochemical substances. Phytochemicals are secondary metabolites that play physiological effects in addition to providing defence and adaptability mechanisms for plants. Phytochemicals include things like alkaloids, flavonoids, phenolics, tannins, terpenoids, saponins, steroids, and glycosides (Harborne, 1998; Evans, 2009). The ability of phenolic and flavonoid compounds to scavenge free radicals has attracted a lot of attention. These phytochemicals are antioxidants that



scavenge free radicals using a variety of techniques (Harborne, 1998; Evans, 2009). Furthermore, terpenoids, alkaloids, tannins, and saponins have all been connected to benefits for health and medicine (Sofowora *et al.* 2013).

The content and composition of secondary metabolites in plants vary greatly between species and can be influenced by a variety of factors, including genotype, plant organs, developmental stages, ambient conditions, season, soil characteristics, and extraction techniques. Comparative phytochemical studies are crucial for understanding the chemical variety of various species and identifying the most promising avenues for further investigation.

Part of the Vidarbha area in Maharashtra, the Wardha region is home to a wide variety of plants, including wild, cultivated, and medicinal ones. Furthermore, the majority of regional plants are used or ingested for medicinal purposes (Shende *et al.*, 2014). In fact, a number of studies have shown how diverse the area is. This study aimed to determine the phytochemical profile of some selected plants from the Wardha region and assess phytochemical contents.

1.1 Objectives

1. To comparatively evaluate the qualitative phytochemical composition of selected indigenous plant species.
2. To determine the occurrence of alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, steroids, and glycosides.
3. To quantify total phenolic content and total flavonoid content in the selected plant extracts.
4. To identify species showing comparatively broader phytochemical profiles and higher TPC and TFC values.
5. To provide baseline information for future phytochemical profiling and biological activity studies.

II. MATERIALS AND METHODS

2.1 Study Area

The current research was carried out with an objective to explore the phytochemical components of some indigenous plant species available in the Wardha district of Maharashtra, India. Wardha district is located in the Vidarbha region of Maharashtra and has tropical climatic conditions. Vegetation in the region includes agricultural lands, grazing grounds, village vegetations, roadside bushes, and scrubs, along with some natural vegetation.

A large number of cultivated, wild, medicinal and indigenous plant species grow in the region. Some of these plant species are being utilized since ancient times for various purposes like nutritional, medicinal, agricultural and ethnobotanical uses. Based on their occurrence and traditional uses in the region, some indigenous plant species were selected for the current study to explore their phytochemical components.

2.2 Selection of Plant Species

Five plant species were selected for comparative phytochemical analysis. These plant species are available in Wardha district and grow both naturally and in cultivation. Selection of plant species was made based on their local occurrence and reported traditional uses. Table 1 below shows the selected indigenous plants species used for the study.

Table 1. Selected indigenous plant species for phytochemical analysis

Sr. No.	Plant species	Family	Common/Local name	Plant part used
1	<i>Aegle marmelos</i> (L.) Corrêa	Rutaceae	Bel	Leaves
2	<i>Azadirachta indica</i> A. Juss.	Meliaceae	Neem/Kadunimb	Leaves
3	<i>Achyranthes aspera</i> L.	Amaranthaceae	Aghada	Leaves
4	<i>Senna tora</i> (L.) Roxb.	Fabaceae	Tarota/Chakwat	Leaves
5	<i>Clerodendrum infortunatum</i> L.	Lamiaceae	Bharangi/Bharnagi	Leaves

2.3 Collection and Identification of Plant Material

Leaf samples of the chosen plant species were collected from their natural habitats or other appropriate localities within Wardha area during the appropriate season. Healthy and disease-free leaves were collected to rule out any variations



due to different ages of the plants and different leaf conditions. The collected samples were identified with the help of taxonomic keys, regional flora, appropriate literature, and available herbarium material.

***Aegle marmelos* (L.) Corrêa**

Aegle marmelos is a medium sized deciduous aromatic tree reaching a height of 8-12 meters. It has an irregular trunk with greyish-brown and rough bark, and armed with straight spines. The leaves are trifoliate, alternate, aromatic and have ovate to lanceolate leaflets. The flowers are greenish-white and fragrant, and occur in axillary or terminal clusters whereas the fruit is large, globose to ovoid, hard-shelled, green when unripe and yellow when ripe, many-seeded with aromatic pulp.

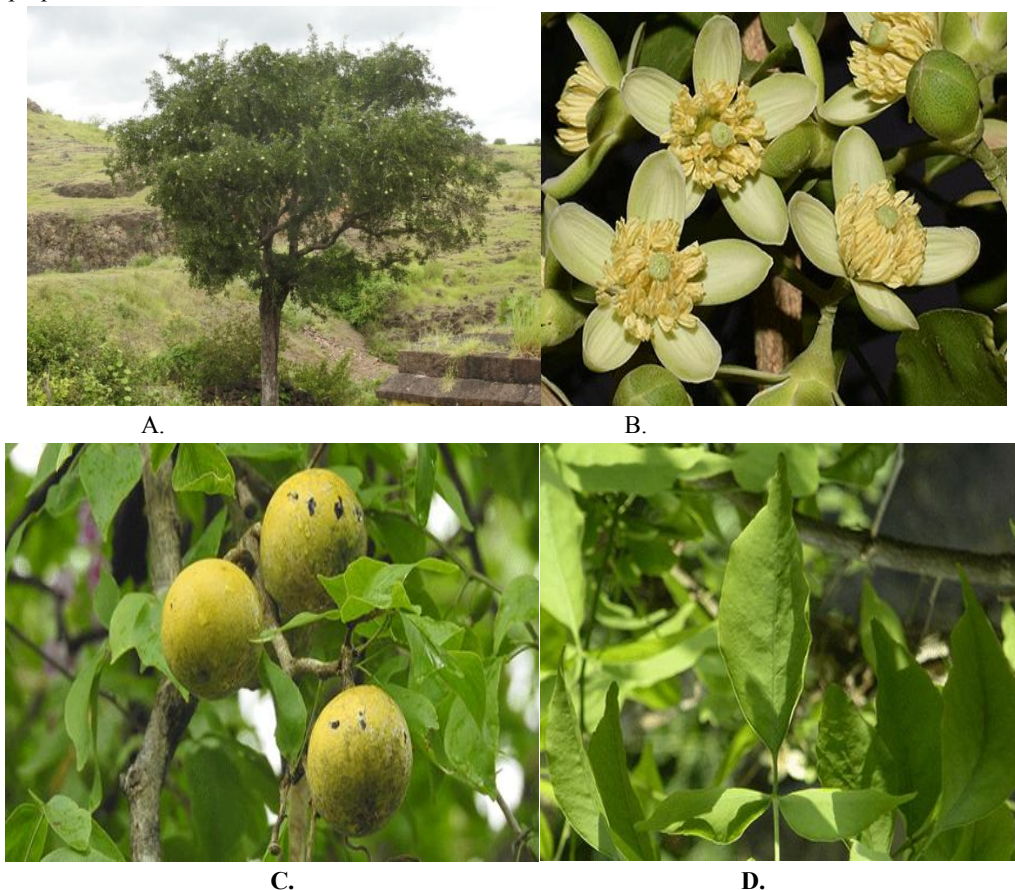


Fig 1: *Aegle marmelos* (L.) Corrêa: A. Habit B. Flower C. Fruit D. Leaf

***Azadirachta indica* A. Juss.**

Azadirachta indica is a fast-growing evergreen to semi-evergreen tree reaching 15-20 m in height. The trunk is straight and dark grey to brown bark. Leaves are alternate, pinnate and compound with many serrate lanceolate leaflets. The flowers are small, white and strongly scented, in axillary panicles. The fruit is a smooth, olive-shaped drupe, green when young and yellow when mature, contain a single seed.



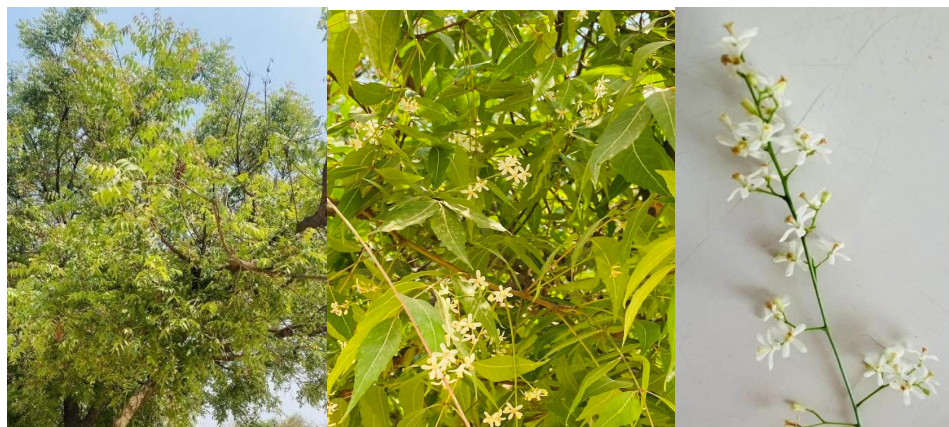


Fig 2: *Azadirachta indica* A. Juss.: A. Habit B. Branch C. Flower

***Achyranthes aspera* L.**

Achyranthes aspera is an erect, much-branched perennial herb, 1-2 m tall. The stems are cylindrical, hairy, and occasionally woody at the base. Leaves are simple, opposite, ovate-elliptic, hairy, and have entire margins. The flowers are small, green-yellow in dense, narrow terminal spikes. The fruits are minutely pubescent, many-seeded, enclosed in persistent bracts and bracteoles that enable them to adhere to animals.



Fig 3: *Achyranthes aspera* L.: A. Habit B. Flower C. Leaf

***Senna tora* (L.) Roxb.**

Senna tora is an annual or short-lived perennial herb, 0.5-1.5 m tall with an erect, branched stem. The leaves are paripinnate, with several pairs of opposite, obovate to elliptic leaflets. The flowers are yellow, in pairs or small clusters in the leaf axils. The pods are slender, sharply curved, cylindrical, and many-seeded with brown seeds. The plant is highly colored and strongly aromatic when bruised.





A. B. C.
Fig 4: *Senna tora* (L.) Roxb.: A. Habit B. Leaf C. Seed

***Clerodendrum infortunatum* L.**

Clerodendrum infortunatum L. is an erect, branched shrub usually 1-2m tall. Stems are somewhat pubescent, with opposite, simple, broadly ovate leaves which have an entire to irregularly toothed margin and distinctive unpleasant odour when crushed. Flowers are showy, white with reddish-purple markings, occur in terminal or axillary cymose inflorescences. The fruit is a globose, four-lobed drupe which turn dark bluish to black at maturity and is surrounded by the persistent calyx.



A. B. C.
Fig 5: *Senna tora* (L.) Roxb.: A. Habit B. Inflorescence C. Leaf

2.4 Preparation of Plant Material

The leaves were cleaned using running water in order to remove dust and other impurities stuck to the surface of the leaf. They were then dried in shade at normal room temperature.



This process was done until a constant weight was attained. The dried plants were separately milled in a clean laboratory mill. The milled samples were sieved and kept in airtight jars that were properly labelled for preservation in a cool and dry place before further processing.

2.5 Preparation of Plant Extracts

Powdered dried leaves (10 g) from each plant were extracted separately using 100 mL of 70% ethanol [Ethanol: Distilled Water, 70:30 (v/v)] keeping an herb to solvent ratio of 1:10(w/v)

Extraction of the mixtures was done by performing intermittent shaking, followed by filtration using Whatman No. 1 filter paper, and concentration under reduced pressure. The concentrated mixtures were poured into labeled airtight bottles and kept refrigerated until phytochemical screening.

2.6 Preliminary Qualitative Phytochemical Screening

A qualitative preliminary study of the phytochemicals present in the 70% ethanolic leaf extracts was performed to establish the existence or non-existence of secondary metabolites such as alkaloids, flavonoids, phenolic compounds, tannins, saponins, steroids, terpenoids, and glycosides. The tests were performed according to phytochemical methods outlined in various scholarly sources (Abubakar & Haque, 2020; Humbare *et al.*, 2022).

For each individual test performed, a suitable amount of concentrated extract was dissolved in the corresponding solvent or reagent mixture. If there is a formation of colour change or precipitation, this will be considered a positive result, while absence of a characteristic reaction will be considered a negative one.

2.6.1 Detection of Alkaloids

Screening of the alkaloids was done by Mayer's, Wagner's, and Dragendorff's tests in accordance with the procedure described by Humbare *et al.* (2022).

Mayer's Test: Approximately 1 mL of the extract was added to Mayer's reagent. Appearance of the white/cream coloured precipitate signified the presence of alkaloids.

Wagner's Test: Approximately 1 mL of the extract was added to Wagner's reagent. Appearance of the reddish-brown precipitate signified the presence of alkaloids.

Dragendorff's Test: Approximately 1 mL of the extract was added to Dragendorff's reagent. Appearance of the orange-red/red precipitate was taken as a positive result for alkaloids.

2.6.2 Detection of Flavonoids

Testing for flavonoids was carried out using the Shinoda test as described by Sood *et al.* (2021). In the test, the extract was mixed with magnesium and hydrochloric acid. Formation of a color that is pink, red, or orange-red indicated the presence of flavonoids.

2.6.3 Detection of Phenolic Compounds and Tannins

Phenolics determination was done through ferric chloride method as described by Sood *et al.* (2021). The appearance of blue, blue-black, or green color was an indication of phenols presence. The screening for tannins was done through ferric chloride. Appearance of dark green or bluish-green coloration indicated the presence of tannins as per Humbare *et al.* (2022).

2.6.4 Detection of Saponins

Saponins were identified by the froth test method based on Humbare *et al.* (2022). The extract was shaken vigorously with distilled water. Frothing was taken as an indication of the presence of saponins.

2.6.5 Detection of Terpenoids and Steroids

Screening of terpenoids was done through the Salkowski test by following the methodology provided by Sood *et al.* (2021). Chloroform solution of the extract was added to concentrated sulphuric acid. The formation of red-brown color at the junction indicated terpenoids.

2.6.6 Detection of Steroids

Steroids / Sterols were determined by Lieberman-Burchard test as per Sood *et al.* (2021). Chloroform, acetic anhydride and concentrated sulphuric acid were added to the extract. Appearance of blue-green / green color development was noted as positive result.



2.6.7 Detection of Glycosides

Cardiac glycosides were tested using Keller-Kiliani test as described by Sood *et al.* (2021). The extract was subjected to a reaction with glacial acetic acid containing ferric chloride and was carefully layered over concentrated sulphuric acid. The appearance of brown or reddish-brown ring was taken as evidence of cardiac glycosides.

2.7 Determination of Total Phenolic Content

TPC was estimated using the Folin-Ciocalteu reagent colorimetry test where gallic acid was used as the reference substance (Singleton *et al.*, 1999). The extracts were reacted with the Folin-Ciocalteu reagent and then sodium carbonate. The absorbance was read on the UV-Visible spectrophotometer after colour generation. There was the presence of a reagent blank.

The calibration graph for gallic acid was used to estimate the TPC in terms of milligram of gallic acid equivalent per gram of dry extract (mg GAE/g). The measurements were done in triplicate and presented as mean $\pm$ standard deviation.

2.8 Determination of Total Flavonoid Content

The total flavonoid content (TFC) was measured via the colorimetric technique using aluminum chloride, and the reference was quercetin. The extracts were treated with aluminum chloride under controlled conditions and their absorbance measured using the UV-VIS Spectrophotometer. A reagent blank was also included in the test.

A calibration curve for quercetin was used to quantify the TFC, and this was measured in mg of quercetin equivalent per g of dry extract (mg QE/g).

2.9 Replication and Statistical Analysis

All phytochemical estimation experiments have been performed in triplicate and data was expressed in the form of mean $\pm$ standard deviation (SD). Descriptive statistics was used for summarizing the variation in total phenolic content (TPC) and total flavonoid content (TFC) in the selected five plants.

Comparison of mean values was done in order to find out the relative amount of TPC and TFC among the selected five plants. In this case no inferential statistical analysis like ANOVA and post-hoc multiple comparison tests were carried out. Thus, variations among species are reported in the form of observed variations without any interpretation of significant difference.

TPC values were reported as milligrams of gallic acid equivalent per gram of dried extract (mg GAE/g) and TFC values as milligrams of quercetin equivalent per gram of dried extract (mg QE/g).

III. RESULTS

3.1 Qualitative phytochemical profile

Qualitative phytochemical analysis showed that there were significant differences in the chemical composition of the 70% ethanol extracts of the five studied indigenous species (Table 2). There were flavonoids, phenolics, tannins, and saponins in all the species under study; thus, it can be assumed that the amount of them in the extract was quite high.

The presence of alkaloids in *A. marmelos*, *A. indica*, *A. aspera*, and *S. tora*, and absence of those compounds in *C. infortunatum* was observed. Similar results were obtained for terpenoids (absence in *C. infortunatum*). In turn, steroids could be identified in *A. marmelos*, *A. indica*, and *S. tora*, and glycosides in *A. marmelos* and *S. tora*.

From the number of positive reactions, *A. marmelos* and *S. tora* are characterized by the highest diversity of phytochemical composition as each of them has eight positive reactions. The third one is *A. indica*, which has seven positive reactions. Six positive reactions are characteristic of *A. aspera*, and four are characteristic of *C. infortunatum* (flavonoids, phenolics, tannins, and saponins).

It is true that the presence of such phytochemicals as phenolics, flavonoids, tannins, saponins, terpenoids, alkaloids, and glycosides in *S. tora* can be attributed to previous researches carried out by Rahman *et al.* (2023) regarding the detection of numerous phytochemicals in leaf extract of *S. tora* using the GC-MS technique. This includes polyphenolics, alkaloids, tannins, and flavonoids. Positive results for phenolics, flavonoids, and tannins in *A. aspera* correspond to those of the study done by Tamta *et al.* (2022) where these phytochemicals were detected in the ethanolic



extract of the plant. Finally, for *C. infortunatum*, positive results for phenolics, flavonoids, tannins, and other phytochemicals correspond to those of the study done by More *et al.* (2024).

However, it should be noted that the qualitative analysis of phytochemicals will give us only the approximate taxonomic composition of the extract. The determination of the exact concentration of specific substances is impossible in this case, and the presence of a reaction may point only to the possible presence of specific phytochemicals. For the confirmation of the results and the determination of the exact composition of 70% ethanol leaf extracts, it is important to conduct such analyses as HPLC-DAD, LC-MS/MS, GC-MS, and NMR.

Table 2. Qualitative phytochemical screening of 70% ethanol extracts of selected indigenous plants

Phytochemical constituent	<i>A. marmelos</i>	<i>A. indica</i>	<i>A. aspera</i>	<i>S. tora</i>	<i>C. infortunatum</i>
Alkaloids	+	+	+	+	—
Flavonoids	+	+	+	+	+
Phenolics	+	+	+	+	+
Tannins	+	+	+	+	+
Saponins	+	+	+	+	+
Terpenoids	+	+	+	+	—
Steroids	+	+	—	+	—
Glycosides	+	—	—	+	—

Note: + = detected; — = not detected.

3.2 Quantitative variation in total phenolic and total flavonoid contents

There was substantial interspecific variation in TPC and TFC among the 70% ethanol leaf extracts (Table 3). While TPC values ranged between 31.68 ± 1.09 and 72.58 ± 1.74 mg GAE/g dry extract, the TFC values ranged between 24.72 ± 0.96 and 89.46 ± 2.05 mg QE/g dry extract.

Azadirachta indica had the highest TPC and TFC values among all the plants tested, with 72.58 ± 1.74 mg GAE/g TPC and 89.46 ± 2.05 mg QE/g TFC respectively. The second highest levels were obtained for *Achyranthes aspera*, where 68.42 ± 1.56 mg GAE/g TPC and 76.35 ± 1.84 mg QE/g TFC were recorded. Intermediate levels of TPC and TFC were found in *Senna tora* with 54.27 ± 1.43 mg GAE/g TPC and 42.86 ± 1.31 mg QE/g TFC.

Comparatively low TPC and TFC values were found in *Aegle marmelos* with 42.36 ± 1.27 mg GAE/g TPC and 38.54 ± 1

Relatively high amounts of TPC and TFC obtained by hydroalcoholic extraction of *A. indica* can be attributed to the recent reports proving the effectiveness of this solvent for extraction of phenolic compounds and flavonoids. The findings about the significant effect of ethanol concentration on the content of phenolics and flavonoids in *A. indica* made by Puttongsiri *et al.* (2025) correlate with the present research results. Nevertheless, there is an inconsistency between the two researches in terms of the actual amounts of TPC and TFC due to different extraction procedure used. It proves the efficiency of 70% aqueous-ethanol extract in extracting significant amount of phenolic and flavonoid compounds from neem leaves.

In addition, relatively high levels of TPC and TFC in *A. aspera* can be considered in light of phytochemical literature review results. Tamta *et al.* (2022) reported substantial amounts of phenolic compounds, flavonoids, and tannins in ethanolic extract of *A. aspera* with antioxidant activity. Even though it is impossible to compare specific results due to different approaches to sample preparation and reporting, it is possible to state that *A. aspera* should be considered as a medicinal plant with a significant content of phenolic compounds.

The TPC of *A. marmelos* was found to be 42.36 ± 1.27 mg GAE/g dry extract in this study. In addition, the value of TPC for *A. marmelos* has been estimated to be 33 ± 7.62 mg GAE/g in a methanolic extract by Kumar *et al.* (2024). Therefore, it may be said that the variation in TPC values is moderate between different types of solvents used. On the other hand, there is a significantly larger variation in the TFC values.



The intermediary values of TPC and TFC of *S. tora* also comply with the general tendency of this species to be phytochemically active. According to Rahman *et al.* (2023), phytochemical compounds were separated from leaf extracts of *S. tora*, among them such groups as phenols, flavonoids, tannins, terpenes, saponins, steroids, alkaloids, and glycosides. It can be stated that the present 70% ethanol extract managed to dissolve flavonoids, phenols, alkaloids, and glycosides.

The relatively low values of TPC and TFC in *C. infortunatum* do not conflict with previously obtained data on the chemical composition of this plant. Previous research has shown that the leaves of *C. infortunatum* consist of phenolic and flavonoid compounds capable of showing antioxidant effects. For instance, according to the results of Verma *et al.* (2025), the content of phenolic compounds is 62.39 ± 1.25 mg GAE/g and that of flavonoids – 42.58 ± 2.05 mg QE/g in the methanolic extract of the leaves of *C. infortunatum*. The difference between the mentioned and currently obtained data might be attributed to differences in the used solvents, extraction yield, geographic location, maturity, and assay conditions.

Table 3. Experimentally determined values of TPC and TFC belonging to the studied plant species.

Plant species	Plant part	TPC (mg GAE/g dry extract)	TFC (mg QE/g dry extract)
<i>Aegle marmelos</i> (L.) Corrêa	Leaf	42.36 ± 1.27	38.54 ± 1.12
<i>Azadirachta indica</i> A. Juss.	Leaf	72.58 ± 1.74	89.46 ± 2.05
<i>Achyranthes aspera</i> L.	Leaf	68.42 ± 1.56	76.35 ± 1.84
<i>Senna tora</i> (L.) Roxb.	Leaf	54.27 ± 1.43	42.86 ± 1.31
<i>Clerodendrum infortunatum</i> L. L.	Leaf	31.68 ± 1.09	24.72 ± 0.96

Note: Values are mean $\pm$ SD (n = 3). TPC = total phenolic content; TFC = total flavonoid content; GAE = gallic acid equivalents; QE = quercetin equivalents.

The relatively higher content of the phenolic and flavonoid compounds in *A. indica* and *A. aspera* may be attributed to the increased availability of these phytochemicals in the leaf extracts of these plants as the experiment was performed in specific conditions. The differences in physiological and biochemical processes in the two species may explain the observed differences.

IV. DISCUSSION

The objective of the current investigation was the comparative determination of qualitative composition and quantitative content of phenolics and flavonoids in the leaves of five local plants. It can be concluded that there are differences between species in both aspects of characteristics.

The maximum values of TPC and TFC have been revealed in *A. indica* leaves. This is confirmed by the content of different secondary metabolites like flavonoids, phenolics, limonoids, terpenoids and other compounds in this plant. The results of Puttongsiri *et al.* (2025) on the effect of the different ethanol concentrations on the content of phenolics and flavonoids in *A. indica* leaves are in accordance with the obtained data. According to the authors, the use of different ethanol concentrations influences on the extraction capacity of the solvent with respect to the target metabolites. That is why the 75% extract contained maximum values of phenolics and flavonoids. In this experiment, 70% ethanol was used, and it can be assumed that the solvent has extracted considerable quantities of polar and moderately polar phenolic compounds.

The second highest values of TPC and TFC were achieved in *A. aspera*. This species had 68.42 ± 1.56 mg GAE/g TPC and 76.35 ± 1.84 mg QE/g TFC, which are almost equal to the values observed in *A. indica*. Tamta *et al.* (2022) studied the ethanolic leaf extract of *A. aspera* and proved its high content of phenolics and flavonoids, as well as pronounced antioxidant, free-radical scavenging, and metal-chelating properties. The qualitative composition of this species is similar, and it also contains substantial amounts of the two groups of polyphenols; therefore, this species is quite promising for further studies.



The phytochemistry of the leaves of *S. tora* is also varied. All eight classes of phytochemicals were identified, while the content of total phenolic compounds (TPC) and total flavonoid compounds (TFC) amounted to 54.27 ± 1.43 and 42.86 ± 1.31 mg/g, correspondingly. In their work on the phytochemical composition of the leaves of *S. tora*, Rahman *et al.* (2023) revealed the presence of phenols, flavonoids, tannins, terpenoids, saponins, steroids, alkaloids, and glycosides. The researchers applied GC-MS analysis for the identification of phytochemical components and showed that the extract had antioxidant, anti-inflammatory, antibacterial effects, and others. Qualitative results of the present research agree with the results obtained by Rahman *et al.* (2023), but quantitative results refute the idea of the abundance of *S. tora* in all classes of phytochemicals.

The leaves of *A. marmelos* were shown to have a relatively rich content of phytochemicals, as the eight tested groups provided positive results. Nevertheless, the content of TPC and TFC was lower than that of the leaves of *A. indica*, *A. aspera*, and *S. tora*. It is also important to emphasize that the qualitative composition of *A. marmelos* cannot be equated to quantitative content. Such an inconsistency is emphasized by the fact that Kumar *et al.* (2024) showed that the leaves of *A. marmelos* have different secondary metabolites (phenolics, flavonoids, tannins), which can act as antioxidants.

Also, it is interesting to note that the leaf extract of *C. infortunatum* was found to be relatively deficient in phytochemicals since only four positive groups were identified during the qualitative analysis. In addition, this plant was found to have the least amounts of TPC and TFC among all tested species. Nonetheless, according to Verma *et al.* (2025), the methanolic extract of *C. infortunatum* leaves is abundant in phenolic compounds and flavonoids and has both antioxidant and anti-inflammatory properties. Moreover, More *et al.* (2024) reported that *C. infortunatum* leaves contain not only phenolics, tannins, and flavonoids but also alkaloids, glycosides, and phytosterols. Thus, different results can be attributed to the difference in sample preparation and testing methods.

4.1 Influence of extraction solvent and analytical methodology

However, caution must be exercised in interpreting any discrepancies between the results obtained from this study and those found in the literature due to the importance of extraction solvent and analytical techniques in such studies. Additionally, there is the need for consideration of several factors, such as ethanol concentration, temperature of extraction, duration of extraction process, solid-liquid ratio, particle size of the material used, drying conditions, and the geographic origin of the species.

For this study, a hydroalcoholic solution with 70% ethanol was employed in order to acquire extracts with abundant polar and moderately polar phytochemicals. It is true that the change in ethanol concentration may influence the solvent's capability to extract various phytochemicals. Thus, the results of this study should only be compared with those achieved from similar extraction solvents and techniques. Furthermore, it is important to point out that the Folin-Ciocalteu procedure is used to quantitatively measure total phenolic content (TPC) through spectrophotometry, and the results indicate the total reducing power of the extract and not the TPC itself. Similarly, the aluminum chloride procedure used to measure the total flavonoid content (TFC) measures the total flavonoid fraction indirectly and does not specifically quantify a certain type of flavonoids. Therefore, the measured values in this study are the total flavonoids, in terms of rutin equivalents.

4.2 Relationship between qualitative and quantitative findings

One of the interesting aspects of this study is that despite having high richness of phytochemicals, there may not be a correspondence between TPC and TFC values. As an example, leaves of *A. marmelos* showed the highest richness of phytochemical classes, while *A. indica* leaves had the highest TPC and TFC values. It can thus be concluded that the amount of phytochemicals is not related to the richness of phytochemicals classes.

The leaves of *A. marmelos* had all the phytochemical groups, but the TPC and TFC levels were the lowest amongst the five varieties. At the same time, the leaves of *A. indica* had seven phytochemical classes, and the TPC and TFC levels were the highest. This is an indication of the disparity between the qualitative test for phytochemicals and the quantitative determination because these results show that different phytochemical classes have different amounts.



4.3 Biological significance

Phenolic and flavonoids are among the most significant groups of secondary metabolites that play a role in giving taste, color, odor, and toxicity to different plant species. The presence of comparatively higher amounts of total polyphenol and flavonoid content in both *A. indica* and *A. aspera* indicates their significant antioxidant property; hence, they deserve to be studied in terms of their pharmacological activity.

The results of this investigation should be viewed as a point of departure for the further investigation of the biochemical properties of the investigated organisms. In this connection, future studies should pay attention to the combination of TPC and TFC tests with DPPH, ABTS, or FRAP tests, among others.

4.4 Regional significance

The present research has a regional significance as the investigated species are native to or can be frequently encountered in the ethnobotanical region of Wardha-Vidarbha. It was proven by previous research on ethnobotanical significance that Wardha and Samudrapur regions have many medicinal plants that are widely used in local traditional medicine. The current investigation brings more insight regarding the phytochemical profile of these plants. The results that prove that *A. indica* and *A. aspera* leaves are the most abundant with total polyphenols and flavonoids content under certain conditions can assist in evaluation of pharmacological significance of these plants. However, before using the obtained results outside of this region, one should take into account the geographical differences.

V. CONCLUSION

The current study investigated the difference in phytochemicals present in five indigenous plants of Maharashtra state, India, that possessed varying qualitative phytochemical profile and total phenolic and total flavonoid content.

Flavonoids, phenolic content, tannin, and saponin were identified in all the plant samples but alkaloids, terpenoids, steroids, and glycosides were identified in some of the plants only. The *Aegle marmelos* and *Senna tora* plants were identified as rich in phytochemicals because all eight categories of phytochemicals were identified in them.

With regard to total phenolic content (TPC) and total flavonoid content (TFC), *Azadirachta indica* showed the highest mean values, being 72.58 ± 1.74 mg GAE/g dry extract for TPC and 89.46 ± 2.05 mg QE/g dry extract for TFC, while the second highest value was shown by *Achyranthes aspera* with TPC of 68.42 ± 1.56 mg GAE/g and TFC of 76.35 ± 1.84 mg QE/g. The remaining plants showed moderate and low values of TPC and TFC.

In view of the current state of scientific research, one can make the assumption that species *A. indica* and *A. aspera* have a higher potential to be beneficial sources of phytochemicals and require further investigations of their phytochemistry. Nevertheless, it is worth mentioning that the current results cannot be generalized for making final conclusions about differences between these species.

Presented qualitative tests and quantitative measurements can be used for preliminary analysis and determination of plant samples phytochemistry. Nevertheless, detected data cannot prove the presence of the certain substance, determine its amount or biological activity. Tests, that were applied, are class specific and the results may differ according to the used material and conditions.

Thus, the analyses performed should cover different methods of chromatography and spectroscopy including HPLC-DAD, LC-MS/MS, GC-MS, NMR, and so on, in order to determine the chemical substances. Moreover, antioxidant tests, antimicrobial tests, etc., should be performed in order to correlate the plant's phytochemistry with the biological activity of the studied plant. Concluding, it should be noted that this study brings out initial findings regarding the phytochemical features of the chosen indigenous plants from the Wardha area. Based on the findings, it becomes possible to conduct more thorough researches, focused on phytochemistry, ethnobotany, and biological properties of these plants.

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