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Phytochemical analysis of *Saraca asoca* bark and *Azadirachta indica* seeds

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Abstract
In view of the prevailing idea of minimizing the adverse effects of synthetic and semi-synthetic compounds through the use of herbal medicine, this study was undertaken to assess the chemical constituents of two important indigenous plants *Saraca asoca* and *Azadirachta indica*. Both plants have found widespread acceptance in herbal medicine, owing to their multitude of effects. Bark of *S. asoca* and seeds of *A. indica* were extracted using methanol and water and their characteristics were studied. Extractability for methanolic and aqueous extracts of *S. asoca* bark was 4.29% and 3.72%, respectively whereas that for *A. indica* seeds was 11.95% and 6.14%, respectively. Phytochemical tests were conducted on these extracts which revealed reducing sugars, tannins, saponins and fixed oils in the methanolic and aqueous extracts of *S. asoca* whereas alkaloids were additional components in the methanolic extract. The methanolic and aqueous extracts of *A. indica* seeds revealed alkaloids, tannins and sterols as common constituents whereas resins and flavonoids were the additional components found in methanolic extract.

Keywords: Phytochemical, *Saraca asoca, Azadirachta indica,* methanolic extract, aqueous extracts

Introduction
Herbal and ethnomedicine has its origin in ancient times when synthetic and semi-synthetic drugs were not available for the treatment of various ailments. Natural remedies were replaced humongously by chemical compounds through the advent of science and technology. However, herbal products still have the potential to become excellent alternatives to chemical compounds owing to their affordability, availability, lack of adverse effects, diversity of ingredients and better stability. Given the wide range of pharmacological effects produced by different herbs, it is essential to know their basic chemical composition for correlation with clinical effects. Moreover, it is necessary to evaluate the effects of these natural compounds separately and in combination on different biological systems.

S. asoca, commonly known as "Ashoka tree", is a flowering plant belonging to the *Fabaceae* family found in evergreen forests of India. However, the plant is also found along the foothills of the Himalayas (Pradhan *et al*., 2009) [1]. The bark of this plant has anti-mutagenic and genoprotective effect (Nag *et al*., 2013) [2], anti-oxidant and anti-breast cancer activity (Yadav *et al*., 2015) [3], anti-bacterial, antioxytocic and antimenorrhagic activity (Panchawat and Sisodia, 2010) [4], as well as anthelmintic activity (Bendigeri *et al*., 2019) [5]. *A. indica*, commonly known as "Neem tree" or "Indian lilac", is a flowering plant belonging to the *Meliaceae* family. The tree is widely distributed across the Indian subcontinent. Neem leaves were first found in Mohenjodaro, Pakistan (Puri, 2005) [6]. Neem leaves have anti-oxidant, anti-tumour, anti-angiogenic and hepato-protective effects (Alzohairy, 2016) [7] as well as the anthelmintic effect (Saiyam *et al*., 2019; Jamra *et al*., 2014) [8, 9]. Neem seeds are a source of neem oil which has anti-inflammatory, anti-arthritic, anti-pyretic, hypoglycemic, anti-ulcer, spermicidal, antifungal, antibacterial and diuretic effects (Bijauliya *et al*., 2018) [10] as well as anthelmintic effect (Kusumlata *et al*., 2017) [11].

Therefore, phytochemical analysis of bark and seeds of these two indigenous plants, *S. asoca* and *A. indica*, respectively, was done to provide a basis for comparison of their chemical composition with *in-vitro* and *in-vivo* effects.

Materials and methods

Collection of plant material: Bark of *S. asoca* and seeds of *A. indica* were collected from the local market of Jabalpur and Veterinary College campus, Jabalpur.

Processing of plant material

Preparation of crude powder: The collected plant material was manually cleaned to remove course impurities and then air-dried in shade at a well-ventilated place in the laboratory. Further drying was done in the incubator to remove moisture at a temperature of 40^0C.The dried bark and seeds were crushed and grounded in electric mixer-grinder to form crude powder and stored in airtight container or poly bags (Azwanida, 2015; Odey *et al*., 2012) [12, 13].

Preparation of methanolic extracts: 50 g crude powder of each plant material was soaked in 400 ml of analytical grade methanol in a glass flask and was covered with aluminum foil followed by stirring at hourly intervals at room temperature (Sharma *et al*., 1971) [14]. Soaking was done for a period of 72 hours. The soaked crude powder was filtered through Whatman filter paper No.1 with separating funnels. The filtrates were concentrated by evaporation at 50-55^{0}C in a rotator vacuum evaporator (Kanojiya *et al*. 2015) [15].

Preparation of aqueous extracts: 50 g of crude powder of each plant material was soaked in 400 ml of triple glass distilled water in a glass flask and was stirred at hourly intervals initially for 2-3 times followed by 8 hours of undisturbed activity at room temperature. Soaking was done for a period of 12 hours. The soaked crude powder was filtered through Whatman filter paper No. 1 with separating funnels. The obtained filtrate was concentrated by using rotator vacuum evaporator at 45-50^{0}C using the procedure described by Kanojiya *et al*. (2015) [15] with some modification.

Preservation of extracts: The extracts were labeled individually and kept in airtight glass bottles in a cool and dark place at 4^0C (refrigerator) for further use.

Calculation of Extractability

Petri dishes were used for evaporation of filtrates obtained during extraction process. Weight of empty petri-dish was recorded as W1. Weight of petri-dish containing the extract after evaporation was recorded as W2. Per cent extractability was calculated using the following formula:

$$\% \text{ Extractability} = \frac{W2 - W1}{\text{Total weight of crude powder used}} \times 100$$

Phytochemical analysis of extracts

Extract residue obtained from each plant were tested for the presence of phytoconstituents such as alkaloids, tannins, flavonoids, saponins, glycosides, resins, triterpenes, reducing sugars and proteins by standard procedures (Das *et al*., 1964; Harborne, 1973) [16, 17].

Test for alkaloids: 0.5 to 0.6 g of various extracts were mixed in 8 ml of 1% HCl, warmed and filtered. 2 ml of the filtrate were treated separately with both reagents (Wagner's and Dragendorff)

a) Dragendorff reagent: The filtrate of the extract was added to the reagent and development of turbidity of precipitation was considered as the presence of alkaloid.

b) Wagner's reagent: The filtrate of the extract was added to the reagent and development of brown flocculent precipitate indicated the presence of alkaloids.

Test for reducing sugars: 5 ml of extract solution was poured in a test tube and equal quantity of Benedict's reagent was added and heated. The appearance of brown red precipitate indicated the presence of reducing sugars.

Test for glycosides: The solution obtained in Benedict's test was filtered and diluted HCl was added. Equal quantity of Benedict's reagent was added and boiled. Appearance of brownish precipitate revealed the presence of glycosides.

Test for tannins: Methanol was added to the residue of the extract. The solution was heated and filtered through Whatman filter paper. Filtrate obtained was treated with different reagents.

a) Lead acetate test: 2-3 drops of lead acetate solution was added to the above mentioned extract solution. The formation of precipitate indicated the presence of tannin.

b) Ferric chloride test: Few drops of ferric chloride solution were added to the above filtrate. A green colouration in the filtrate of the methanolic extract indicated the presence of tannin.

Test for resins: A small amount of extract residue was dissolved in alcohol and a few drops of distilled water were added. The appearance of turbidity was considered as a positive test for resin.

Test for saponins: 1 ml of extract was taken in a test tube and small amount of sodium bicarbonate and water were added. It was shaken vigorously. Formation of froth indicated the presence of saponins.

Test for sterol

a) Salkowski reaction: 1 g residue of extract was taken in 2 ml of chloroform. Thereafter 2 ml of concentrated sulfuric acid was added by the side of the tube. The tube was shaken for a few minutes and the development of red colour in the chloroform layer and greenish yellow fluorescence in the lower layer indicated the presence of sterol.

Test for fixed oil: A drop of aqueous extract was put on filter paper. Appearance of oil base (spot) indicated positive test for the presence of oil.

Test for protein

a) Biuret test: 1 g of residue of extract was taken in water and 1 ml of 4% NaOH solution was added. Appearance of violet pink colour indicated the presence of protein.

Test for Anthroquinone

a) Bontrager's test: A small amount of the extract was boiled for a few minutes with 5 ml of 10% sulfuric acid and filtered immediately while hot. The filtrate was cooled and shaken

with benzene. The benzene layer was separated and shaken with half of its volume of 10% ammonia. The ammonical layer acquiring pink colour indicated the presence of anthroquinones.

Test for flavonoids: 1 ml of extract was dissolved in 5 ml ethanol (95%) and a few drops of diluted NaOH solution was added. Intense yellow colour appeared in the test tube. It became colourless on addition of a few drops of diluted HCl indicated the presence of flavonoids.

Results

The methanolic extract of *S. asoca* bark was brownish black in colour and solid in consistency with an extractability of 4.29%. The aqueous extract of *S. asoca* bark was dark brown in colour and solid in consistency with an extractability of 3.72%. The methanolic extract of *A. indica* seeds was reddish yellow in colour and semi-solid in consistency with an extractability of 11.95%, whereas, the aqueous extract of *A. indica* seeds was yellowish black in colour and semi-solid in consistency with an extractability of 6.14% (Table 01). In phytochemical analysis, methanolic and aqueous extracts of *S. asoca* bark revealed the presence of reducing sugars, tannins, saponins and fixed oils as common constituents whereas the methanolic extract had alkaloids as an additional component (Table 02). Methanolic and aqueous extracts of *A. indica* seed revealed the presence of alkaloids, tannins and sterols as common constituents whereas the methanolic extract had resins and flavonoids as additional components (Table 03).

Table 1: Extractability and physical properties of *Saraca asoca* bark and *Azadirachta indica* seeds

S. No	Physical properties	*S. asoca* bark		*A. indica* seeds	
		Methanolic extract	Aqueous extract	Methanolic extract	Aqueous extract
1	Colour	Brownish black	Dark brown	Reddish yellow	Yellowish black
2	Consistency	Solid	Solid	Semi-solid	Semi-solid
3	Extractability	4.29%	3.72%	11.95%	6.14%

Table 2: Phytochemical analysis of *Saraca asoca* bark extracts

S. No	Active Principle	Test Applied	Result	
			Methanolic	Aqueous
1	Alkaloids	a) Dragendorff test b) Wagner's test	Negative Positive	Negative Negative
2	Reducing sugars	Benedict's test	Positive	Positive
3	Glycosides	Benedict's test	Negative	Negative
4	Tannins	a) Lead acetate test b) Ferric chloride test	Positive Negative	Positive Positive
5	Resins	Hydroalcoholic extract solution in distilled water	Negative	Negative
6	Saponins	Foam test	Positive	Positive
7	Sterols	Salkowski test	Negative	Negative
8	Fixed oils	Filter paper test	Positive	Positive
9	Proteins	Biuret test	Negative	Negative
10	Anthroquinones	Bontrager's test	Negative	Negative
11	Flavonoids	Addition of diluted NaOH solution and diluted HCl	Negative	Negative

Table 3: Phytochemical analysis of *Azadirachta indica* seeds extracts

S. No	Active Principle	Test Applied	Result	
			Methanolic	Aqueous
1	Alkaloids	a) Dragendorff test b) Wagner's test	Positive Negative	Positive Positive
2	Reducing sugars	Benedict's test	Negative	Negative
3	Glycosides	Benedict's test	Negative	Negative
4	Tannins	a) Lead acetate test b) Ferric chloride test	Positive Negative	Positive Positive
5	Resins	Hydroalcoholic extract solution in distilled water	Positive	Negative
6	Saponins	Foam test	Negative	Negative
7	Sterols	Salkowski test	Positive	Positive
8	Fixed oils	Filter paper test	Negative	Negative
9	Proteins	Biuret test	Negative	Negative
10	Anthroquinones	Bontrager's test	Negative	Negative
11	Flavonoids	Addition of diluted NaOH solution and diluted HCl	Positive	Negative

Discussion The extractability of methanolic extract of *S. asoca* bark (4.29%) was in agreement with the report of Tewari *et al*. (2017) [18] who reported an average yield of 4.75% in methanolic extract of cultivated *S. asoca* bark samples whereas in wild samples the yield was less (2.39%). However, our extractability of methanolic extract was in disagreement with the results of Nataraj and Hiremath (2009) [19] who reported an average yield of 13% for ethanolic extract of *S. asoca* bark. This difference in extractability was because their solvent used (ethanol) was different from the solvent used in our study (Absolute methanol). Muregi *et al*. (2007) [20] reported a yield of 4% for *Albizia gummifera* bark methanolic extracts is in agreement with our results of 4.29% extractability for methanolic extract of *S. asoca* bark. The comparable results could be attributed to similar solvent used for extraction as well as the crude relatedness of the two plants.

The extractability of aqueous extract of *S. asoca* bark (3.72%) was in disagreement with the results of Nataraj and Hiremath (2009) [19] who reported lowest yield of 5.92% for aqueous extract but very close to the average yield of aqueous-methanolic extract (1.86%). Possible reason for these differences could be the different method of extraction used and different particle size of the bark powder. The extractability of bark of a related plant *Acacia nilotica* in water was 11 times higher (33.28%) as reported by Patel *et al*. (2015) [21] when compared to that of the aqueous extract of *S. asoca* bark in our study. Similarly, Eguale *et al*. (2011) [22] reported an extractability of 35.6% for aqueous extract of *Albizia schimperiana* bark. This higher yield could be due to greater quantity of crude powder used for extraction (100 g) in both studies. The extractability of bark of another related plant *Adenanthera pavonina* (4.18%) in water as reported by Hussain *et al*. (2011) [23] was comparable to the extractability of *S. asoca* aqueous extract (3.72%). This could be due to common physicochemical properties of the bark of the two plants (*Fabaceae*).

The extractability of methanolic extract of *A. indica* seeds (11.95%) in our study was slightly higher than the extractability (5.61%) reported by Ibrahim and Kiranmai (2012) [24]. This could be attributed to differences in the method of extraction used and the particle size of the seed powder. However, the extractability in our study was close to the extractability of methanolic extract of *A. indica* leaves (10.53%) as reported by Saiyam (2018) [25]. This could be attributed to a similar methodology of extraction. Nathan *et al*. (2006) [26] reported that methanolic extraction of seeds of *Melia azedarach* yielded an oily dark residue which is comparable to the oily, dark reddish-yellow residue obtained in our study. This could be due to the near relatedness of *M. azedarach* and *A. indica* as both plants belong to the same family (*Meliaceae*), same sub-family (*Melioideae*) and same tribe (*Melieae*). The extractability of seeds of *Khaya senegalensis*, which is a related plant, was higher (33%) in methanol as reported by Ayo *et al*. (2007) [27] when compared with that of the methanolic extract of *A. indica* seeds in our study. This could be attributed to the different methodology of extraction used (Soxhlet extraction). The extractability of seeds of a related plant *Swietenia macrophylla* in methanol (15%) as reported by Maiti *et al*. (2007) [28] was comparable to that of methanolic extract of seeds of *A. indica* in our study (11.95%). This was because the method of extraction used was similar to our study.

The extractability of aqueous extract of *A. indica* seeds (6.14%) was less than the extractability of hydro-alcoholic extract (15.25%) of seeds (Ibrahim and Kiranmai, 2012) [24] and aqueous extract (13.55%) of leaves (Saiyam, 2018) [25] but close to the extractability of methanolic extract (5.61%) of seeds as reported by Ibrahim and Kiranmai (2012) [24]. This difference can be attributed to different solvents used, different particle size of powder used, different origin of plants and differences in the methodology. Maiti *et al*. (2007) [28] reported an extractability of 12.7% in water for seeds of *S. macrophylla*, slightly higher than the extractability of seeds of *A. indica* in water (6.14%). This could be attributed to a higher quantity of water soluble components in the seeds of *S. macrophylla* as compared to the seeds of *A. indica*.

The presence of alkaloids in the methanolic extract of *S. asoca* bark could not be demonstrated in the study conducted by Mukhopadhyay and Nath (2011) [29], Mohan *et al*. (2016) [30] and Suja *et al*. (2012) [31]. In our study alkaloids were detected in the methanolic extract in Wagner's test. A variety of reasons could be responsible for this disparity of results such as the test used for detection, different origin of plant material and different stage of plant during collection of bark. Presence of reducing sugars, tannins and saponins in the methanolic extract has been demonstrated by Mukhopadhyay and Nath (2011) [29], Suja *et al*. (2012) [31] and Nataraj and Hiremath (2009) [19]. Therefore, our results were in agreement with their study. Similarly, fixed oils could not be demonstrated in the methanolic extract by Suja *et al*. (2012) [31]. Possible reasons for this disparity could be different detection methods used, different stage of plant during collection of bark and different origin of plant material.

Earlier studies conducted by Divya *et al*. (2017) [32] and Nataraj and Hiremath (2009) [19] have also demonstrated reducing sugars in aqueous extracts of *S. asoca* bark and therefore our results are in agreement. Presence of tannins in aqueous extract has been demonstrated in phytochemical studies conducted by Suja *et al*. (2012) [31] and Pandey *et al*. (2011)[33] and therefore our results are in agreement. However, Divya *et al*. (2017) [32] demonstrated that tannins were absent from the aqueous extract of *S. asoca* bark. Possible reason could be that in the study conducted by Divya *et al*. (2017) [32], only one test viz. the ferric chloride test was used for detection of tannins, which showed negative result. However, in our study we used both tests viz. lead acetate test and ferric chloride test for detection of tannins. Presence of saponin in the aqueous extract of *S. asoca* bark has been demonstrated by Mohan *et al*. (2016) [30], Nataraj and Hiremath (2009) [19] and Divya *et al*. (2017) [32] and therefore our results were in agreement with their studies. Fixed oils could not be demonstrated by Suja *et al*. (2012) [31] in the aqueous extract of *S. asoca* bark. However, in our study fixed oils were detected by filter paper test. This difference in result could be due to different methods of detection used.

Earlier studies by Jafari *et al*. (2013) [34] and Kosma *et al*. (2011) [35] have reported the presence of alkaloids, tannins and sterols in the methanolic extract of Neem seeds. However the latter could not demonstrate the presence of flavonoids whereas the former did. This difference could be due to the difference in method used for extraction. Eddy and Mamza (2009) [36] demonstrated the presence of alkaloids, flavonoids and tannins in the ethanolic extract of *A. indica* seeds, but could not demonstrate resins and sterols, whereas Dubey *et al*. (2014) [37] demonstrated the presence of sterols in the hexane extract of Neem seeds. This difference could be due to different solvents (methanol, ethanol and hexane) used for extraction.

Presence of alkaloids in aqueous extract of *A. indica* seeds has been demonstrated earlier by Lisanti *et al*. (2018) [38]. However, the same author could not demonstrate the presence of tannins and sterols in the extract. Possible reason for this disparity could be different method of extraction or different stage of the plant during collection of seeds. The composition of aqueous extract of *A. indica* seeds was identical to that of *S. mahagoni* seeds. Yasotha *et al*. (2019) [39] also demonstrated alkaloids, tannins and sterols in the aqueous extract of *S. mahagoni* seeds. This can be attributed to the fact that both the plants viz. *A. indica* and *S. mahagoni* belong to the same family i.e. *Meliaceae*. Dash *et al*. (2017) [40] and Saiyam (2018) [25] demonstrated the presence of alkaloids and tannins in the aqueous extract of *A. indica* leaves but could not demonstrate the presence of sterols. This is because the richest natural sources of phytosterols are vegetable oils and vegetable oils are obtained from seeds of plants (Ostlund, 2002) [41].

5. Conclusion

The extractability of methanolic extract for both the plants was higher than that of their aqueous extracts. Both the methanolic and aqueous extracts of *S. asoca* bark had reducing sugars, tannins, saponins and fixed oils as common constituents whereas the methanolic extract had alkaloids as an additional component. However, the methanolic and aqueous extracts of *A. indica* seed had alkaloids, tannins and sterols as common constituents whereas the methanolic extract had resins and flavonoids as additional components.

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