

Original Article

PHYTOCHEMICAL PROFILE, FABRICATION, AND EVALUATION OF HERBAL TABLETS

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ABSTRACT

Objective: The present study investigates the qualitative and quantitative phytoconstituents and to develop and optimize herbal tablets from the methanolic leaves extract of *Xanthium indicum J. Koenig ex Roxb* and their evaluation.

Methods: Preliminary phytochemical screening analyses of methanolic *Xanthium Indicum J.* leaves extract were determined using standard protocol methods led down in the Pharmacognosy textbook by CK Kokate. In addition, formulation of herbal tablets with same leaves extracts by wet granulation technique using various concentrations of adsorbent materials such as starch and methylcellulose. Pre-formulation studies such as bulk density, tap density, carr's index, Hausner's ratio, and angle of repose for each formulation were carried out to optimize the flow properties of the granules followed by compression and evaluation of the tablet.

Results: The results revealed that methanolic leaves extract of *Xanthium indicum J.* was found to be the predominant occurrence of phytochemicals such as alkaloids, cardiac glycosides, carbohydrates, flavonoids, phenol, tannins, saponins, terpenoids, amino acids, and carotenoid. The quantitative estimation of bioactive phytoconstituents showed phenolic compounds 285.75±18.24 mg GAE (Gallic acid equivalent)/ gm of extract and flavonoids 209.52±20.91 mg QE (Quercetin equivalent)/gm of extract. The optimized formulated herbal tablet was selected as per the evaluation parameter concerning Indian Pharmacopoeia.

Conclusion: Methanolic extract of the *Xanthium indicum J.* leaves holds promises as a potential source of pharmaceutically important phytochemicals and the overall study of the formulated tablets containing plant extract suggests that the optimized formulation is now ready for further preclinical study on animals for its biological activity.

Keywords: Herbal drug, *Xanthium indicum J.*, Phytochemical analysis, Preformulation studies, Evaluation parameters

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INTRODUCTION

The oldest type of healthcare known to mankind is Herbal medicine. Throughout history, herbs have been used by all cultures. It was an integral part of the event of recent civilization. Herbal medicinal products are any medicinal products that contain only one or more active substances. WHO reports that 80% of the world's population relies on drugs of natural origin. Several traditional herbal medical practices have been adopted to diagnose, prevent, and treat various diseases. The objective of the development of herbal formulation is to provide synergistic, potentiated, agonistic/antagonistic pharmacological agents within themselves and work together in a dynamic way to produce maximum therapeutic efficacy with minimum side effects [1]. The most important method of administering drugs for systemic effects is the oral route of drug administration. The oral route is the most preferable administration route of Ayurvedic herbal formulations. Designing oral herbal formulations is to date a challenge in modern pharmaceuticals [2]. Tablet and capsule represent unit dosage forms in which one usual dose of the drug has been accurately placed. The tablet has several advantages over the capsule; the tablet is an essentially tamperproof dosage form. The tablet should be a chic product having its own identity while being freed from a defect like chips, crack, discoloration, and contamination and will have the strength to face up to the trials and must have suitable chemical stability over time to not allow alteration of the medicinal agent. The tablet should have the chemical and physical stability to maintain its physical attributes over time [3].

Advantages of herbal medicines include: -

- They have better patient tolerance as well as acceptance.
- The medicinal plants have a renewable source of cheaper medicines.
- Improvements in the quality, efficacy, and safety of herbal medicines with the development of science and technology.

- Prolong and uneventful use of herbal medicines may testify to their safety and efficacy.
- They have lesser side effects as compared to synthetic products.
- The cost of manufacture is quite affordable and is eco-friendly as well [4].

Xanthium indicum J. (local name: ghagra kata) is a folk medicinal plant belongs to the Family Asteraceae [5], widely distributed in different parts of the world such as North America, Brazil, China, India, etc, and has been used traditionally as cooling, fattening, anthelmintic, digestive antipyretic, asthma, rheumatism, leprosy, migraine, smallpox, cancer, etc. The northeast tribal villagers people have been using the different parts of this plant for a long time ago and they claim various Ayurvedic medicinal properties to cure as well as prevent different types of diseases. In Assam, the lower leaf and floral tops are boiled in water and are used as edible plants [6]. *Xanthium indicum* has been reported to be used by the Lohit community of Arunachal Pradesh, India for the treatment of inflammation-related diseases [7]. Keeping this in view, efforts are underway to search for a better understanding of qualitative, and quantitative chemical composition and also to explore the various parameters of the oral formulation.

Wet granulation tablet formulations based on microcrystalline cellulose (MCC) and lactose as binder/diluents are commonly used in the pharmaceutical industry due to their desirable attributes, particularly MCC. It's well-known, however, that lactose could react chemically with drugs containing an amine moiety (particularly primary amine), and so the utilization of lactose as a filler within the formation of these drugs should be avoided. In such cases, MCC might be used alone or could be used together with other fillers like dicalcium phosphate dihydrate and mannitol. The superb compatibility of MCC may well be reduced significantly upon wet granulation [8].

MATERIALS AND METHODS

Chemicals

All the chemicals for phytoconstituents analyses as well as for the development of tablet, tablet excipients such as microcrystalline cellulose (MCC), starch, talc, magnesium stearate, and preservatives methyl paraben, used in the study were of analytical grade and purchased from Loba Chemicals and Merck by Department of Pharmacy, RIPANS, Mizoram.

Collection of plant material and identification

The fresh leaves of *Xanthium indicum* J. Koenig ex Roxb were collected from the west Karbi Anglong in August 2019. It had been identified and authenticated by an expert curator Dr. Souravjyoti Borah, Department of Botany, Guwahati University, Assam where the plant was deposited for future reference. Authentication No is BSI/ERC/Tech/2019/288.



Fig. 1: *Xanthium indicum* J. Koenig ex Roxb

Preparation of methanol extract

Fresh leaves of *Xanthium indicum* J. Koenig ex Roxb collected and gathered leaves were separated from undesirable materials or plant or plant parts. Shade dried the leaves for two weeks. Dried leaves were grounded to coarse powder with the assistance of a mechanical grinder. About 200 gm of powdered material was taken in a very clean maceration chamber and soaked in 900 ml of 80% methanol and kept for fifteen days accompanying occasional shaking and stirring. The entire mixture was then filtered employing Whatman No.42 filter paper. After obtaining clear filtrates, solvent recovery was done with the help of a Soxhlet extractor. Extract concentrated with the help of a water bath and stored in the fridge at 4 °C for further use.

Qualitative phytochemical analysis

The extract of *Xanthium indicum* J. leaves was screened for the presence of phytochemical constituents like alkaloids, phenols, tannins, flavonoids, and steroids following standard procedures [9, 5].

Detection of alkaloids

The crude extract was mixed with a few ml of dilute hydrochloride acid and filtered. The filtrate obtained was carefully tested with various alkaloidal reagents as follows:

Mayer's test: A drop or two of Mayer's reagent were added by the side of the test tube to a few ml of filtrate. The appearance of white or creamy precipitate indicated the test as positive.

Wagner's test: Few drops of Wagner's reagent were added by the side of the test tube to a few ml of filtrate. The appearance of reddish-brown precipitate confirmed the test as positive.

Hager's test: 1 ml or 2 ml of Hager's reagent were added by the side of the test tube to a few ml of filtrate. The appearance of a prominent yellow precipitate indicated the test as positive.

Detection of cardiac glycoside

Baljit test: A drop of Baljit's reagent to 2 ml of the test solution. A yellow-orange color confirmed the presence of glycoside.

Keller-Kiliani test

To 1 ml filtrate, 1.5 ml glacial acetic acid added and 1 drop of 5% ferric chloride followed by a few drops of conc. H₂SO₄ (along the side of the test tube). A blue-colored solution (in the acetic acid layer) confirmed the presence of glycoside.

Test for carbohydrates

Fehling's test

2 ml from an equal volume of Fehling A and Fehling B reagents mixture was added to the crude extract and boiled gently. The appearance of a brick-red precipitate at the bottom of the test tube indicates a positive result.

Benedict's test

Approx. 2 ml of Benedict's reagent mixed with the crude extract and boiled, the appearance of reddish-brown precipitate indicated the presence of the carbohydrates.

Molisch's test

The crude extract was mixed with 2 ml of Molisch's reagent and shaken vigorously followed by the addition of 2 ml concentrated H₂SO₄ carefully along the side of the test tube. The visibility of a violet ring at the interphase indicated a positive result.

Barfoed's test

To 1 ml crude extract filtrate, 1 ml Barfoed's reagent was added and heated for 2 min. The appearance of red precipitate indicated the presence of carbohydrates {monosaccharides}.

Test for proteins and amino acids

Biuret test

To 2 ml extract filtrate, 1 drop of 2% copper sulfate sol. Added along with 1 ml of 95% ethanol and KOH pellet. A pink-colored sol. (in the ethanolic layer) confirmed the presence of protein.

Millon's test

Approx. 2 ml of Millon's reagent was added to the crude extract, the appearance of white precipitate which turned red upon gentle heating confirmed the presence of protein.

Ninhydrin test

The crude extract when mixed with 2 ml of 0.2% solution of Ninhydrin and boiled. The appearance of a violet color suggests the presence of proteins and amino acids.

Xanthoprotic

To plant extract added a few drops of concentrated Nitric acid. A yellow-colored sol confirmed the presence of proteins

Test for flavonoids

Alkaline reagent

To 1 ml extract, 2 ml of 2% NaOH solution and a few drops dil. HCl added. An intense yellow color becomes colorless with the addition of diluted acid which indicates the presence of flavonoids.

Ferric chloride

A few drops of 10% ferric chloride solution were added to the aqueous extract solution. A green precipitate confirmed flavonoids.

Conc. H₂SO₄

To plant extract, a few drops of conc. H₂SO₄. An orange color solution indicates the presence of flavonoids.

Lead acetate

To 1 ml extract, a few drops of 10% lead acetate solution. A yellow precipitate confirmed flavonoids' presence.

Test for phenolic compounds**Ellagic acid test**

To aqueous extract solution, 5% glacial acetic acid and 5% sodium nitrite solution were added. The solution turns muddy/Niger brown precipitate indicating the presence of phenolic compounds.

Iodine test

To 1 ml extract, a few drops of dilute iodine solution were added. A transient red color confirmed phenolic compounds' presence.

Lead acetate test

Plant extract dissolved in 5 ml distilled water followed by 3 ml of 10% lead acetate solution. A blue color at the interface indicates phenolic compounds' presence.

Test for carotenoid

To 1 gm extract, 10 ml chloroform was added with vigorously shaking and filtered. Filtrate mixed with a few drops of conc. H₂SO₄. A blue color at the interface indicates phenolic compounds' presence.

Ferric chloride test

To the aqueous extract solution, a few drops of 5% ferric chloride solution were added. The dark green/bluish-black color confirmed the presence of phenolic compounds.

Detection of tannins**Gelatine test**

Plant extract dissolved in 5 ml distilled water, 1% gelatin solution, and 10% NaCl added thereafter. A white precipitate confirmed the presence of tannins.

Braymer's test

To 1 ml extract filtrate, 3 ml distilled water and 3 drops of 10% Ferric chloride solution added. The blue-green color solution indicates the presence of tannins.

10% NaOH

To 0.4 ml plant extract, 4 ml 10% NaOH was added and shaken well. The formation of emulsion indicates the presence of hydrolyzable tannins.

Detection of quinones**Test for terpenoids**

The crude extract was dissolved in 2 ml of chloroform and evaporated to dryness followed by the addition of 2 ml concentrated H₂SO₄ and heated for about 2 min. The appearance of grayish color indicates the presence of terpenoids.

Saponin

To 0.5 gm plant extract, 2 ml water was added with vigorously shaking. Persistent foam for 10 min confirmed the presence of saponin.

Quantitative phytochemical analysis [10]**Total phenolic content**

The amount of phenol within the methanol extract was determined by the Folin-Ciocalteu reagent method with some modifications. A combination of 2.5 ml 10% Folin-Ciocalteu reagent and 2 ml of 2% solution Na₂CO₃ were added to 1 ml plant extract. Incubation of resulted mixture was done at room temperature for 15 min. At 765 nm wavelength, the absorbance of the sample was measured c. Gallic acid was used as standard (1 mg/ml). All the tests were performed in triplicates. The results were determined from the standard curve and were expressed as gallic acid equivalent (mg/gm of the extracted compound).

Total flavonoid content

The aluminum chloride colorimetric method was used with some modifications to determine the flavonoid content of methanol extract. A known value (1 ml) of sample plant extract was taken. To this 3 ml of methanol was mixed followed by the addition of 0.2 ml of 10% aluminum chloride, 0.2 ml of 1M potassium acetate, and 5.6 ml of distilled water in sequence. And thereby, kept at room temperature for 30 min. The absorbance was measured at 420 nm wavelength with the assistance of UV-visible spectroscopy. Quercetin was used as standard (1 mg/ml). All the tests were performed in triplicates. Flavonoid contents were determined from the standard curve and were expressed as quercetin equivalent (mg/gm of the extracted compound).

Tablet formulation

Tablets were formulated by varying the excipients amount of each formulation as shown in the table below:

Table 1: Selected formulation ratio

Ingredients (in %)	F1	F2	F3	F4	F5
Herbal extract	60	60	60	60	60
Microcrystalline cellulose	35	35	30	30	40
Starch	10	20	15	15	15
Magnesium stearate	0.75	0.75	1	0.75	1
talc	0.5	0.5	0.35	0.5	0.35
Methylparaben	2	2	2	2	2

Preparation of granules**Wet granulation**

The extract obtained was sticky. So, to prepare granules, we have added adsorbents materials such as starch and microcrystalline cellulose to the extract. For the wet granulation, sprinkles of water were done in a mortar and pestle and stirred continuously till a soft wet mass was obtained. The wet mass then passed through sieve mesh size #30 to get uniform granules which were collected on a suitable plate. The granules were then dried in the tray dryer at 50 °C for about 30 min. To the dried granules (fig. 1), calculated

amounts of magnesium stearate, talc, and methyl paraben were added. The formed granules were then undergone pre-formulation study followed by compression to tablets.

Pre-formulation study of granules [11]**The angle of repose**

The angle of repose was tested by the fixed funnel method. The prepared granules of each formulation were poured into a glass funnel respectively. The lower tip of the glass funnel was 5 cm height from the ground. The height (h) and radius(r) of the pile were measured, and then calculated as follows:

$$\tan \theta = \frac{h}{r}$$

$$\Rightarrow \theta = \tan^{-1}\left(\frac{h}{r}\right)$$

Where θ = angle of repose (°)

h=height (cm)

r=radius (cm)



Fig. 2: Sample dry granules of different formulations

Bulk density

The weight of each preparation or formulation was weighed accurately, and gently by pouring it into a 100 ml glass cylinder without compacting. The volume of the powder mixture was recorded and then calculated as follows:

$$\text{Bulk density (BD)} = \frac{m}{V_0}$$

Where, m= mass (g), V_0 = unsettled apparent volume (cm^3)

Tapped density

The glass cylinder with powder mixture from bulk density testing was used to test tapped density. It was tapped using a tapped density tester [Erweka D-6315] for 1,250 strokes. The volume of tapped powder mixture was recorded and then calculated as follows:

$$\text{Tapped density (TD)} = \frac{m}{V_f}$$

Where, m=mass (g), V_f =final tapped volume (cm^3)

Carr's index

It is also known as the compressibility index since it is an indication of the compressibility of a powder. Data from bulk density and tapped density were used to calculate the compressibility index and are as follow:

$$\text{Compressibility index (C. I.)} = \frac{\text{TD} - \text{BD}}{\text{TD}} \times 100$$

It is always calculated in percentage (%).

Hausner's ratio

It is a direct index of ease of measuring the flow of powder. It was calculated as follows:

$$\text{Hausner's ratio} = \frac{\text{TD}}{\text{BD}}$$

Where, TD= tapped density, BD=bulk density

Evaluation of formulated tablets [12-14]

The formulated tablets (fig. 2) were evaluated as per the Indian Pharmacopoeia, 2010.



Fig. 3: Formulated tablets of different excipients ratios

Weight variation

Twenty tablets were selected and the average weight was determined. Then individual tablets were weighed. The individual weight was then compared with an average weight.

Friability

10 tablets were accurately weighed together, and friability was tested using a Roach Friability tester. After 4 min of rotation at 25rpm, any loose dust from the tablets was removed before accurately weighing again. If friability was not more than 1.0%, it was considered acceptable.

Hardness

Also known as tablet crushing strength is the force required to break a tablet in a diametric compression test. For this test, 10 tablets were selected and placed one by one between anvils of hardness tester (Digital tablet Hardness tester). The crushing strength that just causes the tablet to break was reported in Newton (N).

Disintegration time

For most tablets, the first important step toward a solution is to break down the tablet into smaller particles, a process is known as disintegration. The disintegration time of the tablet was determined in phosphate buffer saline (PBS) buffer (pH 7.4) at $37 \pm 0.5^\circ\text{C}$ using a Veego Disintegration Tester.

RESULTS

Phytochemical screening

Preliminary phytochemical analysis of the extract showed the presence of major classes of phytochemicals such as tannins, alkaloids, flavonoids, carbohydrates, cardiac glycosides, tannin, terpenoids, proteins, and amino acids (table 1).

Total phenolic content

The Total Phenolic content of methanolic extract of *Xanthium indicum* was expressed as mg of Gallic acid equivalent per gm of extract and it was found to be 285.75 ± 18.24 mg GAE/gm of extract. The total phenolic contents were calculated using the following linear equation based on the calibration curve of Gallic acid;

$$y = 0.016x + 0.0572, R^2 = 0.9967$$

Where A is absorbance and X is the amount of Gallic acid in μg .

Total flavonoid content

The total flavonoid content was expressed as mg of Quercetin equivalent to per gm of extract and it was found to be 209.52 ± 20.91 gm QE/mg of extract. The total flavonoid contents were calculated using the following linear equation based on the calibration curve of quercetin;

$$y = 0.0007x + 0.0001, R^2 = 0.9956$$

Where A is absorbance and X is the amount of quercetin in μg .

Pre-formulation study

While comparing the granules study result given in table 2 with the reference range [12], we concluded that Formulation 1 (F1) was the

best formulation among all others. Since Carr's index as well as Hausner's ratio of F1 were found within the 'fair' range and the angle of repose was within the 'excellent' range. And as we could notice that all formulations from F2 to F5 had Carr's index and

Hausner's ratio within the 'passable' range and angle of repose within the 'good-fair' range. Hence, all these findings claimed that Formulation 1 has good flowing properties as compared to all of them which does not cause affect the process of tablet punching.

Table 1: Phytochemical screening of *Xanthium indicum J.* leaves extract

S. No.	Tests	Result
Detection of alkaloid		
1.	Hager's	Present
2.	Mayer's	Present
3.	Mayer's	Present
Detection of cardiac glycoside		
1.	Baljit	Present
2.	Killer Killani	Present
Detection of carbohydrate		
1.	Barfoed	Present
2.	Benedict	Present
3.	Fehling	Present
4.	Molish	Absent
Test for proteins and amino acid		
1.	Biuret	Absent
2.	Millon's	Present
3.	Ninhydrin	Absent
4.	Xanthoproteic	Present
Detection of flavonoid		
1.	Alkaline reagent test	Present
2.	Ferric Chloride	Present
3.	Conc. H ₂ SO ₄	Present
4.	Lead Acetate	Present
Detection of phenolic compound		
1.	Ellagic acid	Present
2.	Iodine	Absent
3.	Lead Acetate	Present
4.	Carotenoids	Present
5.	Ferric	Present
Detection of tannin		
1.	Gelatin	Present
2.	Braymer's	Present
3.	10% NaOH	Absent
Detection of quinones		
1.	Detection of terpenoids	Present
2.	Saponification test	present

Table 2: Data of pre-formulation study of different formulation

Studies	F1	F2	F3	F4	F5
Bulk density (g/ml)	0.720	0.675	0.610	0.578	0.563
Tap density (g/ml)	0.880	0.862	0.797	0.770	0.728
Carr's Index (%)	18.18	21.69	23.46	24.93	22.66
Hausner's ratio	1.22	1.27	1.30	1.33	1.29
the angle of repose (°)	25.34	31.75	32.47	36.02	39.28

Evaluation of tablet

Hardness

The hardness of the prepared tablet of each formulation was measured by using a hardness tester (Digital tablet hardness tester). It was recorded in terms of Newton (N) ranging from 10 to 50 N. From the findings, it was found that F1 had the highest hardness of 42.67 N and F5 had the lowest with a reading of 23.22 N.

Friability

While comparing the friability of each formulation as mentioned in table 3, we found that all the formulations are within the range of ± 1 . Hence, we concluded that all the formulations had acceptable friability and no capping problem in a tablet so they could be considered for commercial use.

Weight variation

The physical appearance was a milky brown, smooth and concave tablet. The weights of 20 tablets were measured and it was found to be in the range of 1.0-2.5%. Since as per the I. P. the average percentage weight variation is within ± 5 . Hence, we conclude that all formulations were prepared uniformly.

Disintegration time

It is defined as the time required by the dosage form to break up into granules. The DT of the tablet was determined in phosphate-buffered saline (PBS) buffer (pH 7.4) at $37 \pm 0.5^\circ\text{C}$ using a Veego Disintegration Tester. After experimenting, it was found that all the formulations had a disintegration time of 10 min. While F1 had the highest DT of about 6 min and F5 had the lowest reading of 4.2 min. All the values of different parameters are given in table 3.

Table 3: Evaluation of tablet

Formulation	Wt. variation (%)	Friability (%)	Hardness (N)	Disintegration time (min)
F1	1.7326±0.0021	0.19±0.090	42.67±0.06	6
F2	1.0477±0.0031	0.23±0.075	36.05±0.07	5.5
F3	1.7911±0.0025	0.27±0.081	31.33±0.09	5.1
F4	1.9811±0.0012	0.32±0.051	25.67±0.08	4.6
F5	2.0711±0.0032	0.35±0.082	23.22±0.065	4.2

*All the tests were performed in triplicates and expressed as mean±standard deviation.

DISCUSSION

The present study concludes that the *Xanthium indicum J.* leaves have the potential to act as a source of useful drugs because of the presence of various phytochemical constituents such as alkaloids, flavonoids, phenol, terpenoids, saponin, and carbohydrates. These Phyto-constituents seemed to be the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are a vital role in good health. As there are no or minimal side effects, treatment with medicinal plants is considered very safe [15]. The biggest advantage is these remedies are in sync with nature. The golden fact is that the utilization of herbal treatment is independent of any age group and the sexes [16]. Nowadays, people are shifting from synthetic compounds to herbal products due to the above-mentioned advantages. But for consumption of the herbal extract of herbs, one suitable formulation is required to consume easily. Tablet is a unit dosage form and offers the greatest compatibilities of all oral dosage forms for the greatest dose precision and the least content variability. Tablet's cost is the lowest of all oral dosage forms, easiest and cheapest to package/strip as well as easy to swallow with the least tendency for hang-up. Sustained-release product is also possible by enteric coating and also objectionable odor or bitter taste can be masked by coating technique in tablet formulation. Tablets are suitable for large-scale production and product identification is easy.

CONCLUSION

The overall study of the formulated tablets containing aqueous plant extract suggests that the optimized formulation is now ready for further preclinical study on animals for its biological activity. However, marker compound/s need to be identified to quantify the compound/s through a dissolution test.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

This article does not contain any conflict of interest.

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