

Preliminary physico – phytochemical and phytocognostical evaluation of the leaves parts and evaluation of herbal ointment using leaves of *Achyranthes aspera* L. Leaf extract

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Abstract

Herbal plants are huge sources of nutraceuticals, prevent the different disease or maintain healthy life. Plant produces primary metabolites for their basic survival and secondary metabolites for their ecological, taxonomical and biochemical differentiation and diversity. *Achyranthes aspera* L. (Family Amaranthaceae) are broadly used in traditional system of medicine throughout different part of India, South Andaman Island, Pacific Island, Baluchistan, Tropical Asia, America, and Australia. It has great medicinal importance like to stomach tonic, diuretic, laxative, anthelmintic, anti hyperlipidemic, expectorant, anti-inflammatory, anti-bacterial, anti-fungal, hypoglycemic, anti-asthmatic and anti-allergic etc. The study of diseases and their treatment are important part of our ancient time worldwide. The knowledge of medicinal plants must have been accumulated in the course of many centuries. Herbal medicine prepare different part of plant are used. Herbal drug is design as the alternative formulation for the external use in the form of ointment. For the clinical use the herbal ointment apply externally on human body. The main aims of this research are Preliminary physico-phytochemical & phytocognostical evaluation of the leaves parts and preparation of herbal ointment from the *Achyranthes aspera*. *Achyranthes aspera* L. plants used as antibacterial activity. Under this research work, it is design as herbal ointment with the help of Ethanolic extract of *Achyranthes aspera* and determines the anti-bacterial capacity on it. In vitro study the antibacterial activity of the Ethanolic extracts of dried leaves of *Achyranthes aspera* L. are determine by using the Agar cup plate method versus different bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* etc. By blending the Ethanolic extract of *Achyranthes aspera* L. (10 % w/w) into aqueous cream we formulated herbal ointment. The emulsifying agent and simple ointment bases are added in above herbal ointment for increase the antibacterial effectiveness during in vitro evaluation. The current study deals with the characterization of morphological features, determination of physical constant such as the total ash value, water soluble ash value were 11.25%, 5.25% respectively. Loss of weight drying was 12.9%, foaming index >100, swelling index were 1.5cm, the percent yield for petroleum ether 5.25%, chloroform 6.34%, ethanol 7.65%, and aqueous 8.35%, and determination of formulation and evaluation of herbal ointment using *Achyranthes aspera* L. leave extract.

Keywords: *Achyranthes aspera*; Amaranthaceae; Herbal ointment; *Staphylococcus aureus*; *Escherichia coli*

1. Introduction

Diseases and their treatment now a day's herbal medicine is important part of ancient plant worldwide. Herbal drugs are important resource, especially in developing countries, to treatment of different diseases. India is the largest producer of herbal medicine and it is rightly called the "Botanical Garden of the world". India has number of approved indigenous systems of medicine viz-Ayurveda, Siddha, Unani, and Homeopathy. Naturopathy is applied for the health care of mankind [1]. Herbal plants are effective source of traditional & modern medicines, useful for primary health

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care. This helps to getting increase knowledge of medicinal plants. Ayurveda derived from two words, Au means life and Veda means knowledge. One fourth of modern medicine is derived from plant base origin. Approximately 60% to 80% of the world's populations still depend on traditional medicines for the treatment common diseases. Herbal plants are economical and more acceptable to most of the population in the world. Even in many of the modern medicines, the basic composition is derived from medicinal plants and has become acceptable for easy availability, least side effects, and low prices, environmental friendly and lasting curative property compared to allopathic medicine [2-3]. The World Health Organization (WHO) has defined traditional medicine as “the sum total of all the knowledge and practices, whether explicable or not, also used in diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observation handed gone from generation to generation, whether verbally or in writing” [4]. In one of the studies of the World Health Organization, it is estimated that 80 per cent of the population of developing countries relief on traditional plant-based medicines for their health requirements [4-7]. There are several factors for the continued popularity of traditional drugs and one is their ready availability as compared to the modern medicines besides the adverse effects of synthetic drugs [8]. World Health Organization (WHO) has stressed the need to promote the indigenous systems of medicine among the rural population of the third World Countries [9]. The practice of complementary and alternative medicine is now increase in developing countries in response to World Health Organization directives culminating in several pre-clinical and clinical studies that have provided the scientific basis for the efficacy of many plants used in folk medicine to treat infections [10-11].

Achyranthes aspera L. Under the family amaranthaceae. It is commonly known as apamarga, chirchira and latjira. It is found on road sides, field boundaries and waste places as a weed throughout India up to an altitude of 2100 m and also native to South Andaman Island, Pacific Island, Baluchistan, Tropical Asia, America, Australia [12-13]. *Achyranthes aspera* L. is Stems angular, ribbed, simple or branched from the base, often with tinged purple [12], branches terete or absolutely quadrangular, striate, pubescent leave finely and softly pubescent on both sides, entire, petiolate, petiol [13], flowers greenish white, numerous in axillary or terminal spikes up to 75 cm long, seeds subcylindric, truncate at the apex, rounded at the base, reddish brown. Herbal plants have ability for the formation of secondary metabolites such as steroids, phenolic substances, flavonoids, alkaloids, glycoside etc. These secondary metabolites are used to treatment of many diseases. The secondary metabolites provide a rich biogenic source for novel drug discovery. The metabolites produced by different plants vary from each other. In recent scenario herbal ointment is more popular formulation use for external application. The conveying of drugs through the skin are encouraging concept because ease of access, large surface area, vast exposure to the circulatory and lymphatic networks and protective nature of the treatment [17]. Instead of the alternative formulation like herbal medicine may also be prepared in the form of ointment. These ointment mention a viscous semisolid preparation applied externally on body surfaces area such as the skin, mucus membranes of the eye, vagina, anus, and nose etc. These ointments have specific medicinal values. The medicated ointments contain a medicinal ingredient mixed, suspended or emulsified in the ointment base. Herbal ointment applied externally such as antipruritic, keratolytics, protestants, antiseptics, emollients and astringents. Ointment bases are mainly free from water and generally contain one or more chemical in suspension or solution or dispersion form. Hence Ointment bases may be different types like absorption bases, dehydrating hydrocarbon water soluble type [18]. Its importance for its different traditional uses throughout India, Australia and Kenya. On folklore study *Achyranthes aspera* L. have various therapeutic importances. It is used in the treatment of various diseases such as dropsy, piles, boils, cough and colic in children. *Achyranthes aspera* L. Leaves have been traditionally applied externally to the bite of scorpions. It is small to short sized herb growing to height 1-3 feet. This plant is wild, perennial, erect herb. It is used in treatment of snake bite, ophthalmia and cutaneous diseases. In Uttar Pradesh the plant used as obstetrics, gynecology problem, induction of labor and postpartum bleeding.[15]. *Achyranthes aspera* L. used to treat stomach tonic, diuretic, laxative, anthelmintic, anti hyperlipidemic, expectorant, and anti-inflammatory, anti-bacterial, anti-fungal, hypoglycemic, anti-asthmatic and anti-allergic. Also used as hepatoprotective activity [16]. Today in the modern era, the pathogenic bacteria have developed resistance against existing antibiotics because of the extensive use of antimicrobial drugs against the infectious diseases. So some of the active compounds prohibit growth of the disease causing microbes either singly or in combinations. For a long period of time plants have been a precious source of natural products which are used for maintain the human health, especially in last decades with more extensive studies for natural treatments. There is a continuous and immediate need to invent the new antimicrobials compounds with the varied chemical structure and innovative mechanisms of action for new and re -appearing infectious diseases. So scientists are increasingly turning their attention to community medicines, looking for new leads to develop better drugs against microbial infections. Considering that extracts of *Achyranthes aspera* L. show broad spectrum antimicrobial activity. *Achyranthes aspera* L. are antibacterial activity and also has high potential as antibacterial agent when synthesized as ointment for topical use Ethanolic extract of *Achyranthes aspera* L. no proper report was found regarding and preliminary physico-phytochemical phyto-cognostical evaluation of *Achyranthes aspera* L. till the date. Standardization of herbal drugs are difficult because generally mixture of constituents and the active constituent in most cases is unknown. Now the present study deal the standardize leaves of *Achyranthes aspera* L. Keeping this view the aim of the current study deal the a

Preliminary physico-phytochemical phyto-cognostical evaluation of the leaves parts and formulation and evaluation of herbal ointment using *Achyranthes aspera L.* Leaves extract

2. Material and methods

Leaves parts of *Achyranthes aspera L.* were collected from fields of Itaura, district of Azamgarh, Uttar Pradesh, India in the month of January and authenticated by Prof N.K Dubey, department of botany Banaras Hindu University, Varanasi-221005, and Uttar Pradesh, India. A voucher specimen has been preserved in Department of Pharmaceutical Chemistry, Pharmacy College Azamgarh, and Uttar Pradesh, India for future reference (Voucher specimen no.march-2022-18). The leaves parts were dried under shade and powdered (40 mesh size) and stored in airtight containers.

2.1. Macroscopical studies

The leaves of the plant were studied for their macroscopic characters such as color, odour, taste, shape and size of the leaf. The macroscopic characters were studied as per given procedure in WHO guidelines on quality control methods for medicinal plants materials [19]

2.2. Physicochemical and Phyto-cognostical studies

The loss on drying[20,21], ash value (total ash, acid insoluble ash, water soluble ash)[22-23], foaming index[24], swelling index[19,25], fluorescence analysis[26-30], phytochemical screening[31-33], microscopy[34-35], extractive value (petroleum ether, chloroform, methanol and water), foreign matter were determined according to the official methods of Ayurvedic Pharmacopoeia of India.[20,36-39, Indian Herbal Pharmacopoeia[40]. and the WHO guidelines [19].

2.3. Extraction method

The powdered plant material was extracted with petroleum ether (40-60 °C), chloroform, and ethanol, aqueous respectively in a series using a maceration process. The extracts were concentrated to dryness in vacuum individually to get petroleum ether extract (PEAA), chloroform extract (CEAA), Ethanol extract (EEAA), aqueous extract (AEAA) respectively. The yield of petroleum ether, chloroform, ethanol, aqueous extracts were 5.25, 6.34, 7.65, 8.35% w/w respectively. The extracts are stored in a desiccator. Prepare at different concentrations of Ethanolic extract 500mg/ml, 250mg/ml and 100mg/ml.

2.4. Test microorganisms

The microorganisms used for the study were *Staphylococcus aureus* and *Escherichia coli*. In this study, multi drug resistant wound separates bacteria from pathology, Civil Line, Azamgarh were used. The bacterial strains were raised and managed on Mueller Hinton agar at 37 °C.

2.5. Microbiological media

Chemicals and standard drugs Mueller Hinton Agar and Nutrient broth are collect from the Chemical store of the Pharmacy College, Azamgarh. Gentamicin ointment (1mg of Gentamicin in the form of Gentamicin Sulphate), obtain by medical store of Maha Mrityunjai Hospital, Azamgarh.

2.6. Evaluation of antibacterial activity of Ethanolic Extract (EEAA)

The antibacterial activity of the Ethanolic extracts of the leaves of *Achyranthes aspera L.* (EEAA) at concentrations of 100mg/ml, 250mg/ml and 500mg/ml were determined using the cup plate method. A molten Mueller Hinton agar stabilized at 45 °C was seeded with 0.1 ml of a 24 h broth culture of the test organism (*E. coli* and *S. aureus*) containing approximately 10^8 cfu / ml in a sterile Petri dish and allowed to set. Wells of 6mm diameter were created with a sterile corn borer and filled to about three-quarters full with solutions of the Ethanolic extract of the leaves of *Achyranthes aspera L.* (EEAA).The plates were pre-incubated for 1 h at room temperature to allow for diffusion of the solution and then incubated for 24 h. The zones of inhibition were measured (mean, n=2). Streptomycin and Gentamycin were used as positive and negative controls respectively. The in vitro bacterial response to the extract are evaluate using the diameter of the zones of inhibition as follows; resistant: 10mm and below, intermediate: 11-15mm and susceptible: 16mm and above. [41]

2.7. Preparation of Ointments

Three topical ointment bases of varying degrees of aqueous/anhydrous character namely simple ointment BP, emulsifying ointment BP and aqueous cream BP were prepared by fusion method. In this method the constituents of the base were placed together in a melting pan and allowed to melt together at 70°C. After melting, the ingredients were stirred gently maintaining temperature of 70°C for about 5 minutes and then cooled with continuous stirring. Formulation of ointment done by incorporating 10 g of the semisolid Ethanolic extract of *Achyranthes aspera* L. the various bases by triturating in a ceramic mortar with a pestle to obtain 100 g of herbal ointments containing 10 % w/w of *Achyranthes aspera* L. extract [42]. The prepared herbal ointments were put in ointment tube, labeled and were stored at room temperature.

2.8. Evaluation of ointment

The evaluations were carried out on the ointment by using the following parameters

2.9. Color and odour

Color and odour of ointment, examine by visual examination.

2.10. Loss on drying

1 g of ointment was placed in the Petridis and heated in the water bath at 105 °C every 30 min until it get constant weight.

2.11. pH

The pH of ointment was determined by digital pH meter. 1 g of ointment was dissolved in 50 ml of distilled water and the pH was measured.

2.12. Diffusion study

The diffusion study was carried out by preparing agar nutrient medium of any concentration. It was poured into Petridis. A hole bored at the centre and ointment was placed in it. The time taken for the ointment to get diffused was noted.

2.13. Stability study

The stability studies are carried out for the prepared ointment at temperature of 37 °C for 2 months

3. Results and discussion

The macroscopical study of the leaves of *Achyranthes aspera* L. was done. The leaves were green in color. Short stalked, opposite, elliptic, ovate or broadly rhombate, borne on short petioles (Table-1). The values of the physical constant like ash values, Water soluble ash, foreign matter, loss on drying, extractive value were determined. Extractive value and color of extract was investigated (Table-2). Preliminary qualitative phytochemical screening shown that presence of alkaloids, glycoside, tannins, steroids, flavonoids and saponins (Table-4). Swelling index contain powdered drug 1.5cm. Fluorescence nature of the powder drug & different solvent extracted drug with different chemicals was analyzed using short light wavelength and longer light wavelength and the observation were reported in (Table no-5 & 6). The height of the foam in every test tube was less than 1cm, the foaming index were less than 100 (table-7). The TS of *Achyranthes aspera* L. Leaf showed the vascular strand is shallow, wide and bicollateral. The strand consist of several parallel rows of xylem & Phloem. Also powder microscopy showed trichomes.

The preliminary in vitro antimicrobial activity of the Ethanolic extract of *Achyranthes aspera* L. (EEAA) presented showed excellent activity against *Staphylococcus aureus*. The in vitro antimicrobial activity of the Ethanolic extract of *Achyranthes aspera* L. (EEAA) based herbal ointments. In various case history show that most of the infections are caused by the gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus pyogenes*. Less common cause by the gram-negative bacteria such as *Escherichia coli*. The Ethanolic extract of *Achyranthes aspera* L. (EEAA) leaves showed significant antibacterial activity against all the tested microorganisms. This observation indicates that the activity due to the presence of large varieties of phytoconstituents present in the extract. Hence, the observed antibacterial activities of the ointment are due to the presence of active constituents of the extract and the activity also possess as ointment. This was good sign to do further studies on that to make it as one of the commercial ointment for the treatment of bacterial infections. In literature survey it was found that the plant possesses several traditional and

pharmacological uses. The formulation and evaluation of herbal ointment study of the leaves extract of *Achyranthes aspera* L. were done.

Table 1 Macroscopical evaluation of *Achyranthes aspera* L. leaves

S.NO	Feature	Observation
1.	Color	Greenish
2.	Odour	Characteristic(strong unpleasent)
3.	Taste	Bitter
4.	Shape	Short stalked, Opposite, elliptic, ovate or broadly rhombate, borne on short petioles
5.	Size	Avg. 6-20 mm long

Table 2 Physicochemical analysis of *Achyranthes aspera* L. Leaves

S.NO	Solvent	Weight of plant material (gm)	Percentage of yield (%)	Color of extract
1.	Pet. Ether	4	5.25	Yellowish green
2.	Chloroform	4	6.34	green
3.	Ethanol	4	7.65	Dark green
4.	Aqueous	4	8.35	Brown

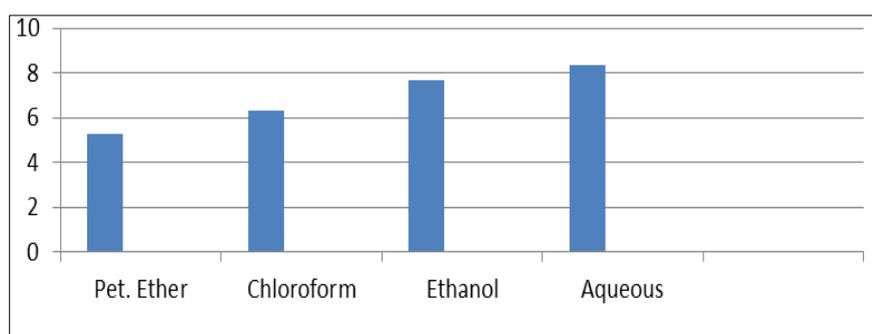


Figure 1 Extractive value (%) different extract of *Achyranthes aspera* L. leave

Table 3 Physicochemical parameters of *Achyranthes aspera* L. leaves

S. No	Physicochemical parameters	Observation
1.	Loss of drying	12.9%
2.	Total cash value	11.25%
3.	Water soluble ash	5.25%
5.	Foreign matter	Nil
6.	Swelling index	1.5 cm

Table 4 Phytochemical screening of *Achyranthes aspera L.* Leaves

S. No.	Test	Pet. Ether extract	Chloroform extract	Ethanol extract	Aqueous extract
1.	Alkaloids	-	+	+	+
2.	Glycoside	+	+	+	+
3.	Cardiac glycoside	+	+	+	+
4.	Terpenoids	+	-	+	-
5.	Flavonoids	-	-	+	+
6.	Steroids	+	-	+	-
7.	Tannins	+	+	+	-
8.	Saponins	-	-	+	+

(+) - Present, (-) - absent

Table 5 Fluorescence Analysis of *Achyranthes aspera L.* leaves Powder

Sl. No.	Treatment	Normal light	U.V. light (Short length)	U.V. light (long length)
1.	Powder + dil HCL	Brown	Light green	Green
2.	Powder +con HCL	yellowish	Dark green	Blackish green
3.	Powder +dil H2SO4	Blackish Brown	Brown	Light brown
4.	Powder +con H2SO4	Brown	Green	Blackish green
5.	Powder + 10%KOH	Yellow	Green	Dark brown
6.	Powder+ Fecl3	brown	Yellow	Dark brown
7.	Powder + dist H2O	Blackish brown	Brown	Light green
8.	Powder + Iodine	yellow	Light green	Dark brown

Table 6 Fluorescence Analysis of *Achyranthes aspera L.* different .leaves Extract

S. No	Extract	Normal light	U.V. light (Short length)	U.V. light (long length)
1.	Pet. ether	Yellowish green	Yellowish green	Black
2.	Chloroform	Dark Green	Dark Green	Black
3.	Ethanol	Dark green	Yellowish Green	Black
4.	Aqueous	Brown	Yellowish Green	Black

Table 7 Foaming index of *Achyranthes aspera L.* different. leaves Extract

Treatment	Sample number of the test tube									
	1	2	3	4	5	6	7	8	9	10
Dilutions(drug extract + water)	1:9	2:8	3:7	4:6	5:5	6:4	7:3	8:2	9:1	10:0
Height of foam(cm)	00	0.1	0.2	0.3	0.3	0.4	0.5	0.6	0.7	0.9

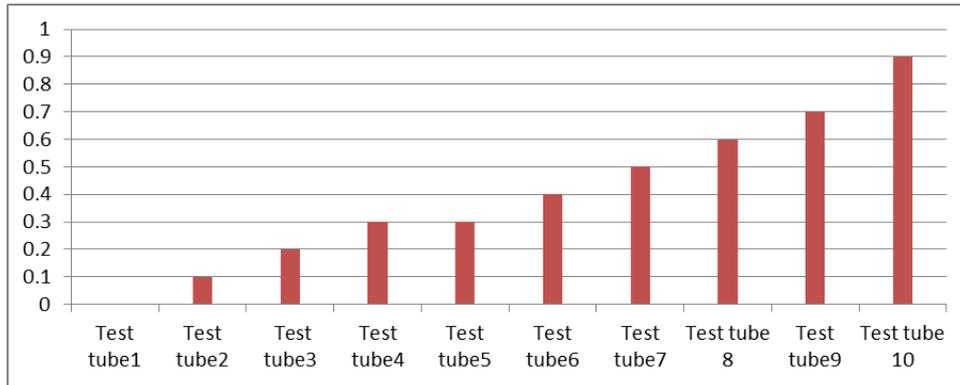


Figure 2 Height of foam (cm)

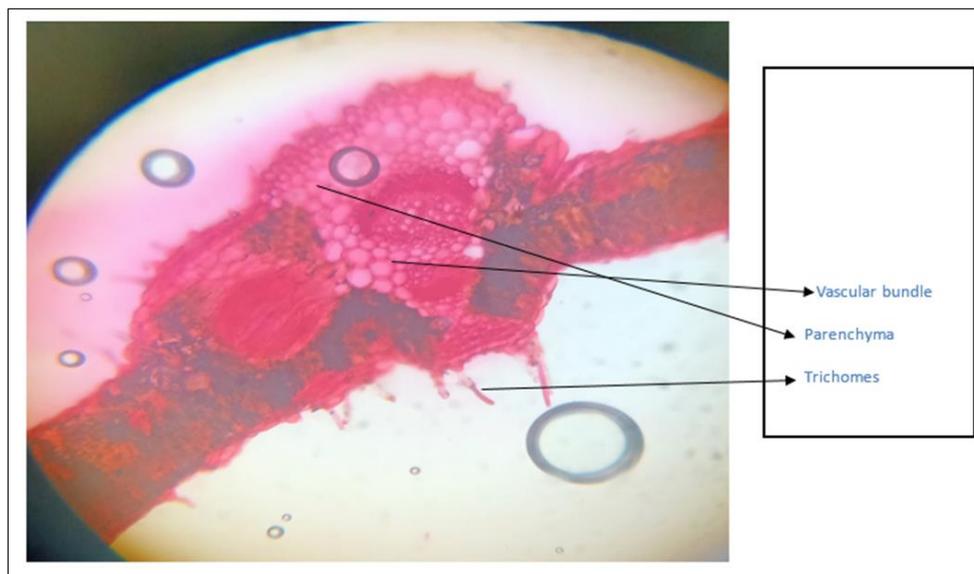


Figure 3 Microscopy of *Achyranthes aspera* L. leaf

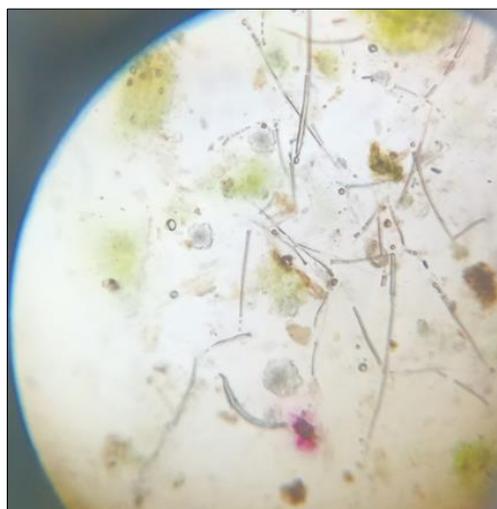
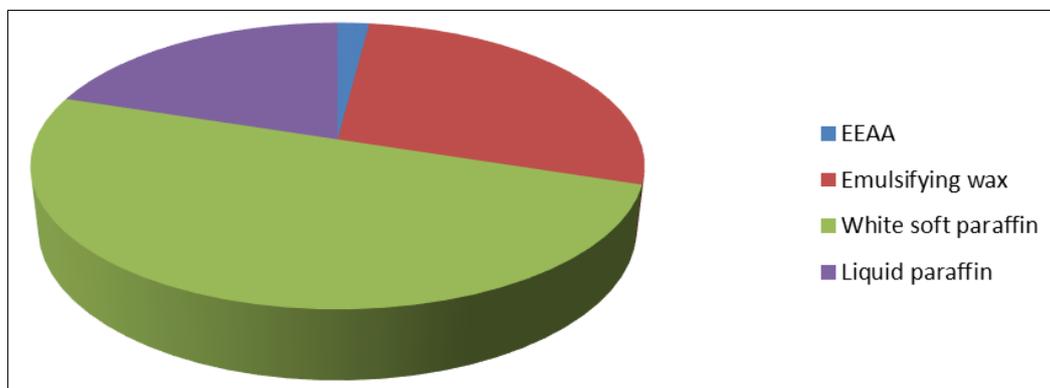


Figure 4 Powder microscopy of *Achyranthes aspera* L. leaves

Table 8 Composition of the Ethanolic extract of *Achyranthes aspera L.* leaves ointment

S. No.	Components	Amount(gm)
1.	Ethanolic extract of leaves Plant <i>Achyranthes aspera L.</i>	2
2.	Emulsifying wax	28
3.	White soft paraffin	50
4.	Liquid paraffin	20

**Figure 5** Composition of the Ethanolic extract of *Achyranthes aspera L.* leaves ointment**Table 9** Physicochemical parameters of *Achyranthes aspera L.* Herbal ointment formulation

S. No	Physicochemical parameters	Observation
1.	Colour	Greenish
2.	Odour and taste	Characteristic
3.	Loss of drying	0.24%
4.	pH	6.13
5.	Diffusion study	2.14 cm in 1 min
6.	Stability study	Stable with pH 6.1

Table 10 Zone of inhibition of the Ethanolic extract of *Achyranthes aspera L.* (EEAA) leaves

S. No	Concentration(mg/ml) of EEAA	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
1.	100	16.63 ± 3.05	15.45 ± 2.00
2.	250	18.67 ± 1.53	16.75 ± 3.06
3.	500	21.06 ± 3.00	19.43 ± 3.05
4.	Ciprofloxacin (5 µg)	28.33 ± 3.05	26.00 ± 2.01

4. Conclusion

Preliminary physico-phytochemical study of the *Achyranthes aspera* L. study concluded to macroscopic, other physical values and parameters will help to identify the species of plant, phytochemical screening will help the presence of compounds, Microscopy is an important tool in the evaluation of crude drugs which is applicable at various levels such as the authentication of the crude drugs, study of powdered drugs, study of T.S. *Achyranthes aspera* L. is known as wide range of medicinal value, it helps to identification, authentication and standardization. In concluded that the present investigation comes out with the fact that *Achyranthes aspera* L. are required so that better, safe and cost effective drugs for treating S. aureus causing diseases. This study shows that *Achyranthes aspera* L. use and could therefore explain the successes claimed in the folk use of the plant in the treatment of common skin conditions. The potency of the *Achyranthes aspera* L. herbal ointment against Staphylococcus aureus could be harnessed in the containment of the organism implicated as the commonest etiologic agent of boils, carbuncles, infantile- impetigo and wound. The final product readily spread on skin surface, showed no irritant effect, diffused well and stable at different temperature. It also require to research on phytochemical and pharmacological aspect. However research going on it would be easier to develop new formula.

Compliance with ethical standards

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Disclosure of conflict of interest

All the authors hereby disclose no conflict of interest.

References

- [1] Anonymous. the wealth of India, Raw material.1985;1A: 423.
- [2] Viji M, Parvatham R, Antioxidant activity and free radical scavenging capacity of in vitro and in vivo regenerated leaf stem and root tissues of *Withania somnifera*-poshita variety, world Journal of Pharmaceutical Research. 2014; 3(2): 4023-4036.
- [3] Hasan P, Yasa N, Ghanbari SV, Mohammad A, Dehghan G, Abdollahi M. In vitro Antioxidant potential of Teucriumnolium as compared to α -tocopherol, Acta pharm. 2007; 57: 123-129.
- [4] The Promotion and Development of Traditional Medicine. WHO Technical Report Series, (WHO), Geneva, Switzerland. 1978; 622: 8.
- [5] In Progress Report by the Director General, WHO, Geneva, Document No. A44/20. 1991.
- [6] Farnsworth NR, Akerele O, Bingel AS, Soejarto DD, Guo Z. Medicinal plants in therapy. Bulletin of WHO. 1985; 965.
- [7] Akerele O. WHO guidelines for the assessment of herbal medicines. Fitoterapia. 1992; LXIII: 99.
- [8] Akerele O. Medicinal plants and primary health care: an agenda for action. Fitoterapia. LIX. 1988; 355.
- [9] Bannerman R, Burton J. Traditional medicine in healthcare coverage, WHO, Geneva. 1983; 1-342.
- [10] Vijaya K, Ananthan S. microbiological screening of Indian Medicinal plants with special reference to enter pathogens. J Altern Complement Med. 1997; 3: 13-20.
- [11] Dilhuydy JM. Patient attraction to complementary and alternative medicine(CAM): a reality which physicians can neither ignore nor deny. Bull Cancer. 2003; 90: 623-628.
- [12] Anonymous. The Wealth of India - Raw Materials, Council of Scientific & Industrial Research, New Delhi, 2005, 55-57.
- [13] R.K. Gupta. Medicinal & Aromatic Plants. CBS publishers & distributors, 2010, 190.
- [14] R. Zafar. Medicinal Plants of India. CBS publishers & distributors, 2009, 1-15.

- [15] Indian herbal pharmacopoeia, volume-II, a joint publication of regional research laboratory & Indian drug manufacturer and association, 1999; 1-8
- [16] A.R. Bafna, S.H. Mishra. *Ars Pharmaceutica*, 2004, 45(4), 343-351.
- [17] Sukanya MK, Shimi, Aruna SR. Phytochemical Analysis Antimicrobial Analysis Antimicrobial careening and Anthelmintic properties Of *Phyllanthus emblica*. *International Journal of Pharma and Bio Sciences*. 2013; 4(4): 55-64.
- [18] Bhandari PR, Kamdod MA. *Emblica officinalis* (Amla): A review of potential therapeutic applications. 2012; 6(4): 257-269.
- [19] A World Health Organization, Geneva; Quality Control Method for Medicinal Plant Materials, A.I.T.B.S. Publisher and Distributors., New Delhi, 2002; 8-24.
- [20] Pharmacopoeia of India, Ministry of Health and family Welfare, Govt of India, New Delhi, Vol-II, 1996, A-53, 54, 89, 95.
- [21] The Ayurvedic Pharmacopoeia of India, part-I, Vol-III 1st edition Ministry of Health and family Welfare, Govt of India, Dept of Health, New Delhi, 2001, 234-235.
- [22] Bhatia D, Gupta M. K, Gupta A. M and Kaithwas J. *Nat Pro Rad* 2008; 7:326.
- [23] Anonymous. Indian Pharmacopeia, Volume 1, The Indian Pharmacopoeia commission, Ghaziabad. 2010; 82-201.
- [24] World Health Organization, Quality Control Method for Medicinal Plant, England, 1998, 28, 30, 46, 76.
- [25] Wallis TE. Text book of pharmacognosy 5th edition CBS publishers and distributors, New Delhi, India. 2005.
- [26] Weltering E. J & Van Doon W. G : role of ethylene in senescence of petals morphological & taxonomical relationship *journal of experimental botany* 1988, 39, 1605-1616.
- [27] Kashi A.R, Ramachandran S, & Sukumaran B. industrial pharmacognosy, university press, 2012, 25.
- [28] Indian Pharmacopoeia, publication and information directorate New Delhi, A-54, 89.
- [29] Kokate C.K. practical pharmacognosy 1st edition, Vallabhprakashan New Delhi, 1986.
- [30] Chase C.R and Pratt R.S, fluorescence of powdered vegetable drugs with particular reference to development of a system of identification, *journal of American pharmacology association*, 1949, 38, 32.
- [31] Khandelwal K.R, practical pharmacognosy technique & experiments, ninth edition, Nirali prakashan, 2002, 149-156.
- [32] Trease and Evans W.C pharmacognosy, 15th edition, 2005, 253-254.
- [33] 33. Mukherjee P.K, quality control of herbal drugs, 1st edition, 2002, 247-378.
- [34] Wallis T. E, textbook of pharmacognosy 5th edition CBS publishers and distributors, New Delhi, India, 1985, 234-236.
- [35] Khandelwal K.R, practical pharmacognosy technique & experiments, ninth edition, Nirali prakashan, 2002, 82-97.
- [36] The Ayurvedic Pharmacopoeia of India, Part-1, Vol:II, (Govt. of India, Ministry of Health and Family Welfare), New Delhi.
- [37] India Pharmacopoeia, controller of publication, Delhi, Vol-I, 1996, 209-210.
- [38] World Health Organization, Geneva; Quality Control Method for Medicinal Plant Materials, Geneva, 1998, 9, 22-24, 33.
- [39] Pharmacopoeia of India, Ministry of Health and family Welfare, Govt of India, New Delhi. 1996; II.
- [40] Anonymous. Indian Herbal Pharmacopeia, Volume 1, Indian Drug Manufacturers Association, Mumbai. 1998; 179-197.
- [41] Singh J, Kaur S. *Phyllanthus embilica* Leaves Extract a Potential Amylase enzyme Inhibitor with antioxidant and antimicrobial activity. *International Journal of Pharmacological Research*. 2019; 5(9): 200-206.
- [42] Guideline 2.1.-Laboratory methodologies for bacterial antimicrobial susceptibility testing. OIE Terrestrial manual. 2012; 1-11