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ANALYSIS OF PHYTOCHEMICALS AND INVASIVENESS OF *XANTHIUM STRUMARIUM* HAVING ETHANOMEDICINAL AND PHARMACOLOGICAL ACTIVITIES

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ABSTRACT

Xanthium strumarium L. is an emerging invassive weed rich in phytochemicals diversity and wide range of pharmacological and ethnomedicinal uses. Several morphological, physiological factors and defence mechanisms that ranging from physical barriers including cuticle formation, lignification, spines and trichomes to the biosynthesis of toxic compounds contributes to its invasiveness. Phenols and tannins play significant role in several beneficial biological activities still act as as herbivore deterrents, and left allelopathic influence on other plants. In this investigation quantification total phenols, bound phenol, ortho dihydroxy phenol, tannic acid, flavanol, quinines, acid detergent fiber and lignin, acid and water soluble ash, crude fibre content is being done. Role of morphological and physiological factors responsible for making this plant invasive discussed in details. Potential benefits of this invasive weed are, photochemical diversity and wide range of pharmacological and ethnomedicinal uses. The plethora of bioactive compounds make it highly significant genus from biochemical and pharmacological point of view. Apart from many drawback of spreading new invasive weed specie, it is also enriching the gene pool of the existing plants and Gene banks with desirable genes. In future it may act as source of important genes contributing in resistance towards biotic stress for plant diseases, pests as well as for abiotic stress resistance.

Keywords: biodiversity, herbivore deterrents, morphological, physiological factors**.**

Introduction

The concept of weed originated when man first started to grow plants for food, hence undesired plants were eradicated from fields. The need for weed control got extraordinary importance as technological advances prevailed. Weeds were either brought by different invaders inadvertently or through seed import. In the first three decades of the 20th century, a number of weed species that were most frequent and harmful disappeared completely or almost completely due to cleaning crop seeds by machines, better and deeper soil tillage, better fertilization, denser crop stands, crop rotation, and herbicide application (Šarić Taib, 2011). Many present day weeds (being alien and invasive) did not exist in the wilderness some years back. *Xanthium strumarium,* going to be one of most noxious and emerging weed of crop lands, widely distributed in North America, Brazil, China, Malaysia and hotter parts of India (Marwat, 1990; Marwat *et al.,* 1993). It is the most studied medicinal plant having phytochemical diversity and ethnomedicinal and pharmacological activities but with toxicity principle. It is rich in phytochemical diversity and reported to have antibacterial, antitumor, anticancer, antifungal, anti-inflammatory, antinociceptive, antititussive, hypoglycaemic, antimitotic, antitrypanosomal, antimalarial, diuretic, antioxidant, analgesic, repellent and insecticidal activities (Kamboj and Saluj 2010, Ganie *et al.,* 2014) .

Secondary metabolites such as alkaloids, terpenoids, phenolics, cyanogenic glycosides, diterpenes and minor chemicals are of vital importance in providing protection against harmful insect, bacteria and fungi and even vertebrate herbivores (Furlan *et al.*2010). Phenolics and tannins are synthesized in plants not only under genetic determinants, physiological demands and evolution-controlled defence needs (Bernays *et al.,* 1983) but also by the influence of environmental stress such as disturbance, drought, and electromagnetic radiation (Miguel *et al.,* 2007). Tannins, polyphenolic secondary metabolites of plants, have both beneficial and adverse function according to their concentration and chemical structure. Phenolics and their oligomeric and polymeric relatives (tannins) continue to receive attention for their regulatory functions in intra-plant, herbivore-plant and pathogen- plant relationships, and also because of their beneficial effects to human and animal health (Lempa *et al.,* 2004; McNally *et al.*, 2003; Shahidi, 2004; Skerget *et al.,* 2004). The mono- or polyaromatic character of phenolics imparts a strong filtration power of against ultra-violet (UV) light (Krauss *et al.,* 1997). Tannins in forages have often been described as antinutritional factors. Lignins are a fraction of cell walls that are indicators of the toughness of plants.

In the present study an attempt is being made to quantify phytochemical which contributes to *X. strumarium* invasiveness and role of morphological and physiological factors responsible for making this plant invasive discussed in details. Comprehensive account on total phenols, bound phenol, ortho dihydroxy phenol, tannic acid, flavanol, quinines, acid detergent fiber and lignin, acid and water soluble ash, crude fiber content is being generated . This invasive weed is an important members of ecosystems enriching biodiversity and gene pools, sustainable utilization of gene of this species might assist in improving some species or cultivars of cultivated plant. It may be of vital importance in the future in crop breeding by genetic engineering as sources of desirable genes to be transferred for attaining resistance against plant diseases, pests, etc.

MATERIAL AND METHODS

Plant Material

Several Plants of *X. strumarium* were collected fresh in bulk from village Neeri Mankar 25 km away from Nagpur. Specimens collected were identified by the authors, one of whom is in charge of the herbarium at Department of Botany, RTM Nagpur University, Nagpur where Herbarium specimen with voucher number RTMB 5879 was deposited. The leaves, roots were collected separately from plant dried under shade was then powdered using mechanical grinder.

Histochemical analysis

Histochemical analysis of cell wall for presence of ergastic inclusions and deposition of suberin, cutin and lignin as well as for the presence of oxalic acid and citric acid crystals and mucilage was carried out following standard methods (Khandelwal, 2007; Bendre and Kumar, 2014**)**.

Quantification of Phytochemicals

Estimation of total phenols and ortho-dihydric phenols, bound phenols and quinines was done subsequently as per Bray and Thrope (1954), Mahadevan and Sridhar (1986) Estimation of tannic acid was done as per reference (Sadasivam, and Manickam 1992) while flavonols content estimated as per reference (Thimmaiah 1999). Estimation of lignins was done by gravimetric method given by Official Methods of the Analysis of the AOAC whereas determination of acid detergent fibres and acid detergent lignin was done separately. Plant ash was prepared (A.O.A.C, 1975) at 400^{o}C and various ash values were estimated following Kulkarni and Apte (2000).

Results

Transverse section of stem and root reveals dense content of ergastic inclusions and high deposition of suberin, cutin and lignin. Histochemical analysis confirms the presence of oxalic acid and citric acid crystals and mucilage also.

Ash yield was found to be higher 35.5 % of dry tissue in leaves which while in stem acid insoluble ash percentage is higher 7.75 % . Water soluble ash is high 31.9% in leaves where as high crude fibre content reported in stem 28.10 % (Table 1).

Among all plant parts, fruits are rich in total phenol 8.65 mg/g, ortho-dihydroxy phenol 2.16 mg/gm and acid detergent fibre 79%. Stem show higher tannic acid content 14.47 mg/gm, flavonols 1.97 ug/gm, and quinines 0.132 ug/gm. Leaves content high levels of bound phenol 4.6 mg/gm and acid detergent lignin (Table No:2). Statistical analyses (ANOVA) of phenol content, for effective comparison across different tissues tabulated in table 3.

Discussion

Weedy and invasive species cause economic loss in crop by changing the availability of nutrients, disturbing pollination and causing the disappearance of indigenous species but also ecosystem function by changing energy and nutrient flows, as well as physical factors within habitats and ecosystems [Levine 2003 *et al.*,]. Biological mechanisms of invasive plants responsible for their success remain poorly understood. Many species have defence mechanisms that range from physical barriers including cuticle formation, lignification, spines and trichomes to the biosynthesis of toxic compounds such as alkaloids and tannins (Delessert *et al.*, 2004).

Survey of literature revels that Physiological characters like high germination and survival rates, rapid growth, unisexual flowers occurring in clusters on branches and main stem, wind-pollination, self-compatibility and self-pollination (Beckett *et al.*,1988,), prolific seed production and dispersion (Redosevich and Holt, 1984), 80 % seeds viability [80 %], somatic polymorphism (Weaver and Lechowicz, 1982) , throughout the year seeds germination (Lawrence, 1986) contributes to invasiveness of *X.strumarium.* Presence of minute bristles on stems and broad leaves make it unpalatable to all classes of livestock. *X. strumarium* induces intoxication and lethal to cattle, sheep, pigs and human (Scherer *et al.*, 2009). This property saves them from blousing by animals thus help in perpetuation and expansion of their area. Its spiny fruit clinging to the wool of sheep/goats has been the major force of its spread.

Transverse section of stem and root reveals dense content of ergastic inclusions and high deposition of suberin, cutin and lignin. Histochemical analysis confirms the presence of oxalic acid and citric acid crystals and mucilage also. The presence of blotched purple colour of stem indicative of anthocyanin pigmentation while woody nature is because of high lignin content. Lignin is a polymer of phenylpropanoid compounds formed through a complex biosynthesis route, represented by a metabolic grid and a component of the plant cell wall. The functional significance of lignin is associated mainly with the mechanical support allowing plants to stand, with water transport in the xylem vases and as a defence against pests and microorganisms (Boudet, 2000). An increase in lignification is often observed in response to attack by pathogen and believed to represent one of a plethora of mechanisms designed to block parasite invasion. It reduces the susceptibility of the host, since lignin is a non-degradable mechanical barrier for most microorganisms. Lignins are cell wall fraction which can be estimated as acid detergent fibre and acid detergent ligninis. Presence of high amount of lignin, Acid detergent fibre, cellulose and silia in all the plant parts give added advantage to the plant.

Secondary compounds synthesized through vegetal secondary metabolism, or, by special metabolism and associated to plant-environmental interactions (Monteiro *et al.,* 2005 and Haslam, 1995). As per theory of ecological apparentness , among five classes of chemical compounds (tannins, alkaloids, phenols, quinines and triterpenes), Phenols and tannins were outstanding in all plant species and habitats, possibly through their widespread distribution, especially among lignified plants (Almeida *et al.,* 2005). The literature about tannins, polyphenolic secondary is vast and often conflicting. Tannins are a heterogeneous group of polyphenolic polymers of varying molecular weight and complexity. Plant phenolics and tannins are synthesized to meet ordinary physiological demands but also as a response to biotic and abiotic stresses (Alonso-Amelot *et al.,*2007).

Traditionally, tannins have been described as modulators in plant-herbivore interactions and protection agents against infection, with the main function as herbivore deterrents due to their acid taste and the property of precipitating proteins. Tannins have been characterized as a form of quantitative defence against insect herbivores (Moilanen & Salminen, 2008) . Surfactants in insect-gut fluids inhibit tannin-protein interactions. Most of the lepdopteran larvae have basic-gut (pH 9-12), which ionizes tannins and results in a loss of hydrogen-binding capacity . Products of tannin oxidation can deplete insect herbivore nutrients or produce cytotoxic effects (Appel 1993, Hagerman *et al.*,). Probably high content of tannin in *Xanthium strumarium* provides immunity against several species of insects except cerambycid beetle, *Apagomerella versicolor* (Boheman) (Guillermo *et al.*,2002) . Despite much research, the mechanisms by which tannins exert their effects on animal health and performance are not completely understood. In addition, the use of different standards for determination of tannins often makes it impossible to compare results between studies.

Phenolic compounds are widely distributed throughout the plant kingdom and range from simple molecules such as phenolic acids to complex polymerised compounds (i.e. polyphenols) (Rice-Evans *et al.,* 1996). *Xanthium* is a reservoir of phenols. The identified phenolic compounds in *X. strumarium* includes , chlorogenic and ferulic acids, thiazinediones, triterpenoid saponin, CAT, xanthanolide sesquiterpene lactones (8-epi-xanthatin and 8-epixanthatin epoxide), several xanthanolides, beta-sitosterol, strumasterol (C-24 epimer of stigamsterol), monoterpene and sesquiterpene hydrocarbons, caffeic acid, 1,3,5-tri-O-caffeoyl quinic acid, 1,5-di-Ocaffeoyl quinic acid (Scherer *et al.*,2009). Phenolic compounds may affect the plant nutrient environment by distinct mechanisms, due to their chemical reactivity. While low-molecular-weight-phenols (LMWP) reduce soil nutrient availability , Phenolic-rich plants negatively modify neighboring plant growth by restricting N supply and also exhibits allelopathic influence of these compounds (Inderjit and Foy 2001) Phenol -rich root inputs could be an unappreciated factor structuring plant communities, especially in N-limited systems dominated by phenol-rich species themselves (Meier *et al.,* 2007). Catechin was a powerful allelochemical, responsible for the adaptive advantage of the invasive species spotted knapweed (*Centaurea stoebe*) (Chobot *et al.,* 2007).

Other Potential benefits of this invasive weed are., photochemical diversity and wide range of pharmacological and ethnomedicinal uses but with Toxicity principle. Phenols have been related to several biological activities (antiviral, antioxidant, diuretic, antirheumatic and others, Gutierrez *et al.,* 2008). On the other hand, tannins are use against diarrhoea, as antiseptics, vasoconstrictors, antimicrobial and antifungal, due to their astringent activity (Falleh *et al., 2008,* Brandelli 2009).

Conclusions

Weed invasion has been under serious debate for about three decades in the world. Alien and exotic plant invasions are threatening the floral diversity around the globe and affect ecological processes. The origin of invasiveness of *X. strumarium* find traces in lack of natural predators in addition to their adaptive nature in alien environments, prolific reproduction, ease to pollinate, high seed output with staggered dormancy and its allellopathic in nature as well. Phenols and tannins are synthesized in plants not only through genetic determinants, physiological demands and evolution-controlled defence needs, but also by the influence of environmental stress such as drought, UV-B radiation and atmospheric pollution. Potential interactions between tannins and other primary and secondary plant compounds are of importance for palatability and biological function. Their functions in plant- interactions depends, to a large extent, not only on the chemistry of these compounds, but also on the strategies that herbivores possess for dealing with these substances. This knowledge will be of importance for sustainable utilization of this invasive spice particularly owing to its phytochemical diversity. The plethora of bioactive compounds make it highly significant genus from biochemical and pharmacological point of view. Apart from many drawback of spreading new invasive weed specie, it is also enriching the gene pool of the existing plants and Gene banks with desirable genes. In future it may act as source of impotant genes contributing in resistance towards biotic stress for plant diseases, pests as well as for abiotic stress resistance for hardiness towards low or high temperatures, drought, salty or flooded soils, etc. Further research is necessary to understand invasive nature of this plant and the mechanisms by which phenols and tannins exert their effects and performance on other plant and animal health.

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(Tables & Figures)

Table 1: Table showing results of proximate analysis of *Xanthium strumarium* plant parts.

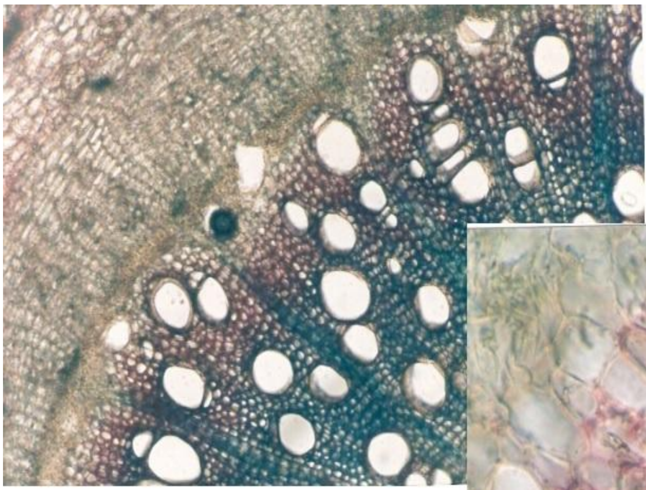
	Dry matter %	Moisture %	Ash %	Acid insoluble ash content %	Water soluble ash content %	Crude fibre content %	Acid detergent fiber %	Acid detergent lignin %
Leaf	42.25	57.75	5.3	3.4	31.9	11.15	22.4	5.3
Stem	46	54	22.15	7.75	14.4	28.1	75.4	1
Root	31.18	68.82	9.75	1.6	8.15	16.95	64.2	10.5
Fruit	34.2	65.8	3.95	1.1	2.85	36.4	79	0.8

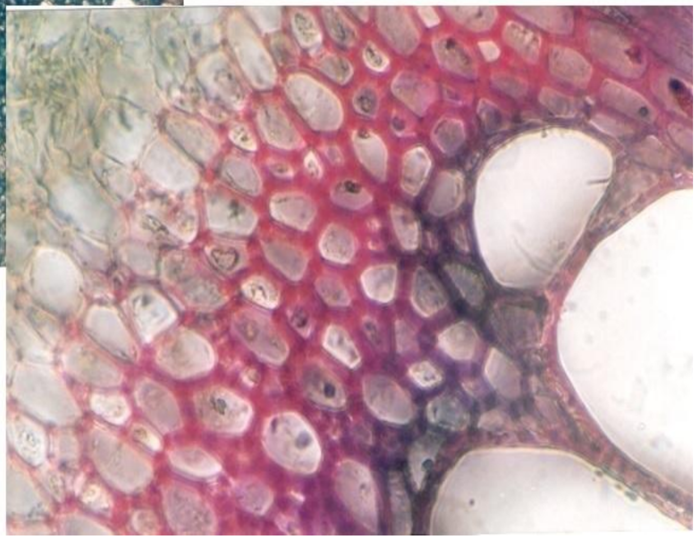
Table 2: Quantification of phytochemicals in plant parts *of Xanthium strumarium.*

Name of phytochemical compound	Total Phenolic Content mg/g (dry weight)	Bound Phenol Content mg/g (dry weight)	Orthodihydroxy Phenol Content mg/g (dry weight)	Tannic acid mg/g (dry weight)	Quinines ug/g (dry weight)	Flavonols Ug/gm at A_{500}
Leaf	2.28	4.63	1.407	12.05	0.046	1.22
Stem	2.76	3.22	1.913	14.77	0.132	1.97
Root	1.7	3.23	1.7	9.98	0.002	0.9
Fruit	8.65	4.28	2.16	13.42	0.054	3.34

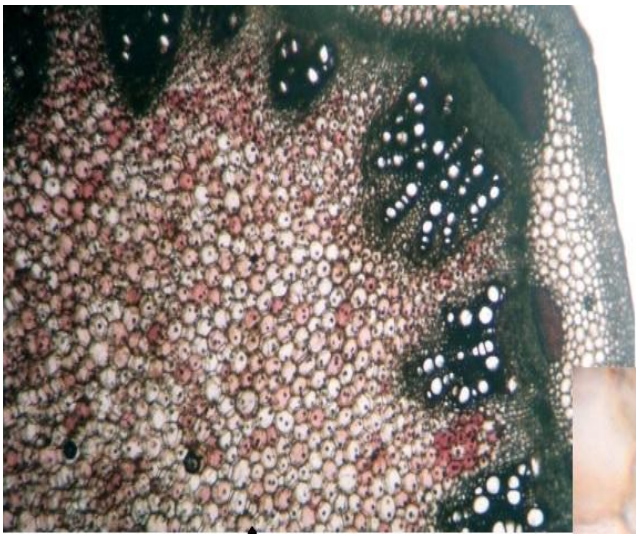
Table 3: ANOVA Table for Phenol Content

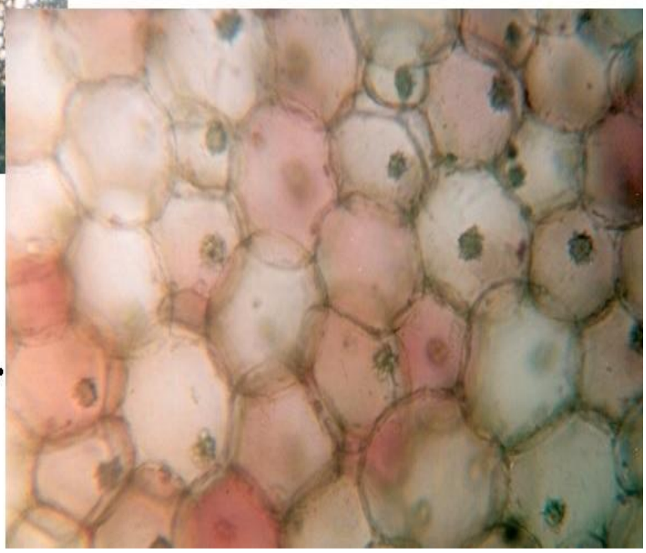
Source of Variation	Degree of Freedom	Sum of Square	Mean Square	Computed F
Total phenol ANOVA cv= 28.96%				
Treatment	15	894.585	17.84	60.067
Error total	32	9.518	0.297	
Bound phenol ANOVA cv=2.83%				
Treatment	15	5.432	0.1306	1.2036
Error total	32	1.959	0.1085	
Orthodihyroxy phenol ANOVA cv = 72.179%				
Treatment	15	16.91	1.127	4.282
Error total	32	8.739	0.273	





Transverse section of root reveals dense content of ergastic inclusions and high deposition of suberin, cutin and lignin





Transverse section of stem at reveals dense content of ergastic inclusions and high deposition of suberin, cutin and lignin