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Isolation and characterization of bioactive pentacyclic saponin from *Xanthium strumarium* L

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Abstract
A pentacyclic triterpene saponin molecule has been isolated from the air-dried plant powder of *Xanthium strumarium*, commonly known as Cocklebur. Air-dried plant powder of *x. strumarium* fruits after defatting with petroleum ether was extracted successively with methanol, chloroform and carbon tetra chloride and partitioned between water and n-butanol. This repeated extraction yielded a saponin molecule, which was identified by TLC and purified by repeated silica gel open column chromatography and further characterized on the basis of HPLC and several spectral techniques such as ^{1}H NMR and ^{13}C NMR, FAB-Mass Spectroscopy and Infrared spectroscopy. Isolated saponin was evaluated for bioefficacy against a major weed of wheat crop *I. rogusum*.

Keywords: Isolation, identification saponin; *Xanthium strumarium*

Introduction
The saponins are a pharmacodynamic group of natural product with a broad spectrum of biological activities (Francis *et al*. 2002) [9] and widely distributed in nature (Vincken *et al*. 2007) [35]. These are commonly found in more than ninety plant families and among them triterpenoid saponin constituted the major group. The Saponins possess detergent-like properties e.g., positive froth test due to their amphiphilic character. Saponins also known to exhibit several biological activities such as anti inflammatory (Anan, 2001) [3], moluscicidal (Kohda *et al*. 1989) [13], antiviral (Simoes *et al*. 1999) [30], pesticidal (Hosttetmann and Wolfender, 1999) [8], Fungicidal (Lucca *et al*, 2000), Cytotoxic (Marquina *et al*. 2001; Fattorusso *et al*. 2000) [16, 11], antidiabetic activity (Badre *et al*. 2000) [5] and bioherbicidal (Sondhia and Saxena 2002, 2004) [27, 26] and other biological activities (Osbourn *et al*.. 2011) [22]. Saponin of *Panax ginseng* plant are well known for its biologically activity (Zhang and Liu, 1994) [40]. Several bioactive saponin molecules have been routinely isolated from the Asteraceae family (Scott *et al*. 2004; Sattar, 2001; Viturio *et al*. 1998) [33, 32, 36] and other plant families (Apers *et al*, 1999; Yoshikawa *et al*. 2000; Marquina *et al*. 2001; Navarro *et al*. 2001) [4, 38, 16]. Sakai *et al*. (1999) [31] isolated a triterpenoid saponin from the ground part of *Ater ageratoides* var. ovatu. Ouyang *et al*. (2001) [21] isolated a triterpenoidal saponin from the leaves of *Ilex kudincha*. Yang *et al*. (1999) isolated eight bioactive triterpenoid saponin from the seeds of *Aescules chinenses*. Simonet *et al*. (1997) isolated a new saponin from the seeds saponin fraction of *Trifolium secupinatum*. Navarro *et al*. (2001) [16] isolated seven-oleane type triterpene saponin from the methanolic extract of the aerial part of *Bupleurum rotundifolium* and identified them on the basis of spectral data.

Xanthium strumarium is a weed of Asteraceae family and considered among the most competitive annual weed (Adcock *et al*. 1990, 1991) [2, 1]. Its fruits are the source of medicinal drug. It showed potential biological activity such as insecticidal (Cetinsoy *et al*. 1998) [6], nematicidal (Nandal & Bhatti, 1990) [19], antifungal (Zacchino *et al*. 1998) [39] and antitrypanosomal properties (Talakal *et al*. 1995) [34]. It competes with soybean and cotton and showed allelopathic potential against lettuce and mungbean (Waller *et al*. 1992) [37]. The objective of our work was to isolate, identify and characterized the biological active molecule from this plant and to evaluate its bioherbicidal activity for the control of other weeds. Plants discharge saponins from the rhizosphere as allelopathic agents so as to suppress the growth of adjacent plants (Moses *et al*. 2014) [17]. In this study, in this study the bioherbicidal activity of crude pentacyclic saponin was evaluated against *Iscahemum rugosum*, since these compounds appear to have a role in plant defense system (Sondhia 2003; Lin *et al* 2009) [25, 14] and play a regulatory role in interactions of plants with other plants.

Material and methods

The whole mature plant of *X. strumarium* was collected in the month of October-November, from Jabalpur, India and dried under shade and powdered. Triterpene saponin molecule was isolated as described by Sondhia and Saxena, (2002) [27]. The process of isolation yielded a white amorphous powder having Rf value of 0.88 on TLC and it was further confirmed to be a triterpene by the Libermann-Burchard chemical test.

Hydrolysis was done with 10% H_2SO_4 at 100 ºC for 6 hours. Hydrolyzed saponin (aglycone) was methylated and methanolysed with 3% methanolic hydrochloride at 80 ºC for six hours followed by neutralization with silver carbonate. The ethyl acetate fraction comprises of pure aglycone was designated as compound I (aglycone fraction); whereas the aqueous fraction comprises of the sugars hydrolyzed from saponin and was designated as compound II (glycone fraction. These two fractions were subjected for spectral analysis. The saponin molecule was elucidated by IR (Shimadzu 8201 PC instrument having a range of 4000-350 cm^{-1}), ^1H NMR, ^{13}C NMR (Bruker DRX 300 MHz FT- NMR) and Mass Spectroscopy (JEOL SX-102 /DA-6000 FAB-MS/data system using Argon/Xenon (6 KV) as the FAB gas).

Bioherbicidal activity) of X. strumarium

Evaluation of bioherbicidal activity of the pentacyclic triterpene saponin isolated from *X. strumarium* was evaluated against *Ischaemum rogusum*. Isolated crude saponin (0.92 g) was dissolved in 2 ml methanol and 20, 10, 2.5 and 1.25% concentrate were prepared by adding distilled water and distilled water served as control. Bioassay was performed as our already established method (Sondhia 2007). Twenty seeds of *I. rogusum* or *V. sativa* were placed on a Whatman filter paper No. 5 in a hundred mm diameter petridish supported by a thin layer of sterilized absorbent cotton. Ten ml of desired concentration of crude saponin extracts were added and petridishes were kept in seed germinator for 72 hours bioassay at 25 ºC $\pm$2 and at 80% humidity. The data on root elongation and shoot growth were taken thereafter. The experiment was replicated thrice.

Results and discussion

Structural elucidation by various spectral studies

The saponin molecule had a melting point of 260^{0}C. The IR peak at 3600-3250 cm^{-1} and 1050 cm^{-1} indicating hydroxyl group and one absorption bands at 1650 cm^{-1} indicating double bonds. Further it showed an absorption band at 3600-3400 cm^{-1} due to the carbohydrate moiety. The prominent peak of 3200-3400 cm^{-1} (for carbonyl OH) and strong signal at 1720 cm^{-1} due to carbonyl absorption, 2950, 1640 for (C=C) and 1465, 1380, 1050 cm^{-1} for (C-C, C-H) tallied well with the olefinic acid ring structure. The number of anomeric carbon in a saponin was determined by ^{13}C NMR and the number of individual sugar residue present by comparing the chemical shift with appropriate model sugars (Seo *et al*. 2002). The ^{13}C NMR spectra of saponin molecule showed signal at 89.3 shifted downfield by 10 ppm as compared to oleanoic acid due to glycosylation shift, indicating the C-3 sugar linkage and suggesting that sugar is linked through C-3 hydroxyl of aglycone. The ^{13}C NMR values of saponin molecule obtained in pyridine were 38.4, 27.6, 90.5, 38.8, 18.1, 32.6, 39.2, 47.8, 36.8, 23.5, 122.7, 144.1, 42.1, 25.8, 23.1, 46.5, 41.0, 45.9, 30.6, 33.8, 32.5, 27.6, 15.3, 16.2, 17.1, 25.8, 180.0, 33.2, 23.5 (C1-C30), Sugars 103.3, 73.3, 78.9, 68.7, 71.4, 61.4, 61.7 (C1-C6), 100.9, 71.6, 72.7, 68.2, 71.0, 62.3 (C1′-C6′).

^1H NMR values of saponin molecule obtained in D_2O, δ ppm were viz 0.81 (9H, s), 0.85 (3H, s), 1.08 (3H, s), 1.15 (3H, s), 1.25 (3H, s) 5.5 (1 H, b, s, CH=C-), and 4.4 (1 H, d, glu) together with ^{13} C NMR indicated that the aglycone belongs to the oleanolic type pentacyclic triterpene ring (Doddrell *et al* 1974) [7]. Consequently the remaining ^1H and ^{13}C NMR signals corresponding to the carbinolic hydrogen and carbon atoms were used to identify a beta –D-glucopyranosyl moiety at C-3. Further 1 H NMR spectrum indicated the presence of three anomeric protons in the sugar moiety at d 4.32(1H), 5.01 (1H) and 4.42 (1H). The D- configuration of sugars were determined by physical data (co-preparative TLC) and confirmed by comparing with authentic sample of D-glucose as well as by comparing the chemical shift values with values from the literature (Doddrell *et al*. 1974) [7] The interglycosidic linkage between sugars was deduced to be Glu (1→3) Glu from the deshielding of one of the CH units of this moiety in the ^{13}C NMR spectrum of compound 1 (δ 78.9) (Figure 1).

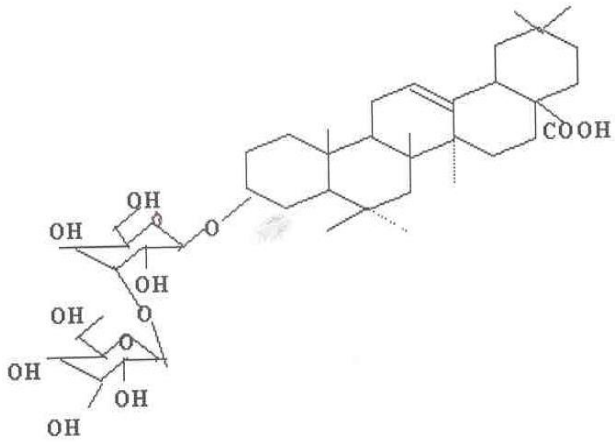

Fig 1: Pentacyclic saponin from *Xanthium strumarium* L.

The positive ion Fast Atom Bombardment- Mass Spectra (FAB-MS) of the whole saponin molecule exhibited the following fragmentation pattern. The fragment ion m/e 248 as the base peak in mass spectrum. Characteristic peaks at m/z 248 and 208 are the results by the fragmentation of ring C, and are the most common peak of oleanane ring skeleton and indicated that the double bond is present in ring C at 12-13 positions. There was a subsequent loss of methyl group (m/z 233) is also obtained ion the mass spectra reported. Peak at 179 obtained due to the loss of hexose moiety and peak at 553 showed a rearrangement type of ion. This pattern of peaks indicated that one carboxylic group is present in ring E (Rahman *et al*. 2000) [23]. The positive ion peak of FAB-MS of compound 1 revealed quasimolecular ion at m/z 781 (M+H) correlate with the formula $C_{42}H_{68}O_{13}$ and peak at 618 and 456 attributed to the fragment loss of one glucose and one galactose units respectively. This structure is designated as 3-O- [beta-D-glucopyranosyl- (1→3) –beta-D-glucopyranosyl (3 beta)-3-hydroxy olean-12-en-28-oic acid with molecular weight 780 and molecular formula $C_{42}H_{68}O_1$.

Bioherbicidal activity of *Xanthium strumarium*

Allelopathic potential is also a characteristic feature of saponin isolated from plants (Sondhia and Saxena 2002) [27]. Alfalfa saponins possess high allelopathic potential against plants, fungi and microorganism (Oleszek, 1996). Our bioassays show that *X. strumarium* has inhibitory effects on

V. sativva seedlings. All the saponin dilutions bioassays showed significant differences at probability 0.05 level as compared with control. Similar results were obtained by Waller *et al.* (1995) where soya saponin I, and II inhibited the growth of mungbean that was growing for 72 hours. The present results demonstrated that *X. strumarium* saponin significantly inhibited root and shoot growth *I. rogusum.* The effect of *X. strumarium* saponin on *I. rogusum* is presented in Figure 2 and 3.

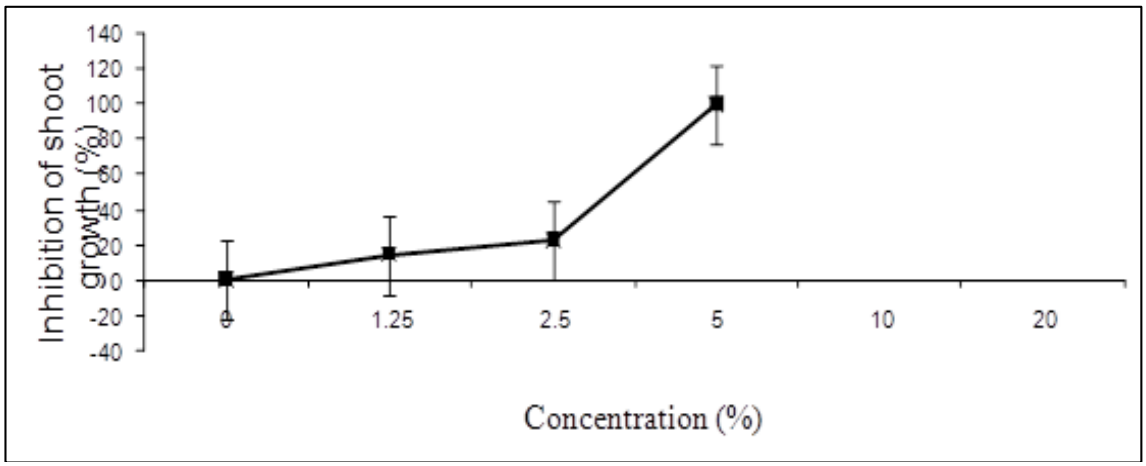

Fig 2: Effect of *Xanthium strumarium* crude saponin on the shoot growth of *I. rogusum*

Significant results were obtained at all saponin concentration. In all the weed seedlings decreasing trend in shoot growth and root growth were observed and it was higher at higher concentration as might be expected. From figure 1 and 2 it clearly appeared that all the concentration of saponin extracts inhibited the root and shoot growth of *I. rogusum* seedlings.

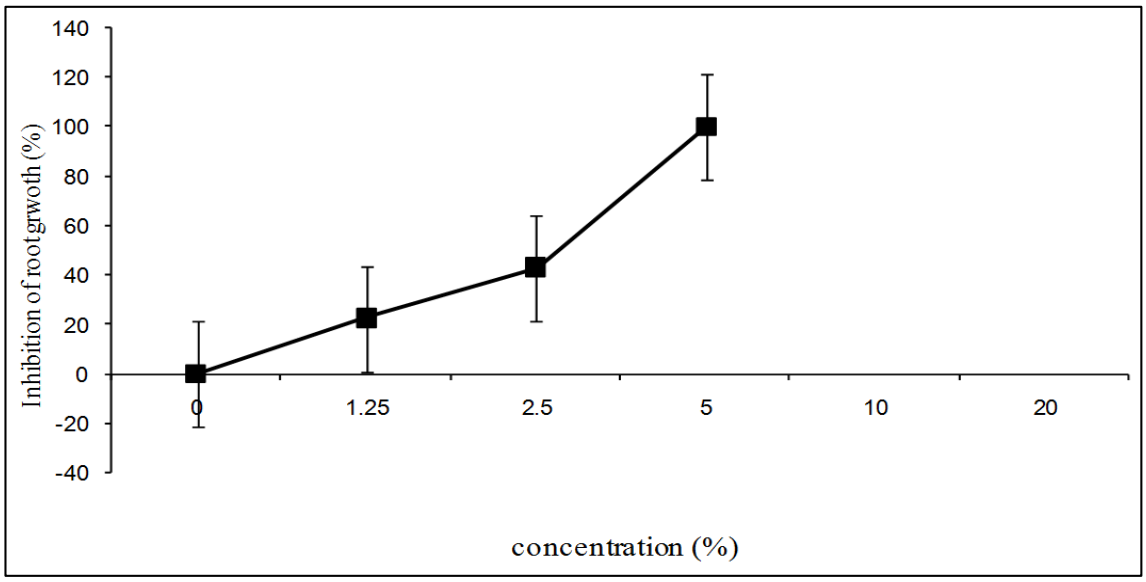

Fig 3: Effect of *Xanthium strumarium* crude saponin on the root growth of *I. rogusum*

Root growth was affected more severely than shoot growth in all the tested weed seedlings (Figure 1 and 2). Further, 5% crude saponin of *X. strumarium* completely inhibited the growth of *I. rogusum* seedlings (root and shoot) as compared to control. The inhibitory activity of pentacyclic saponin isolated from the *X. strumarium* showed that it has potential to inhibit growth of *I. rogusum* at 5% saponin concentration, and may be used as a potential herbicide template.

Conclusion

Xanthium strumarium saponin strongly inhibited the growth of *V. sativa* and its inhibitory effect This is comparable with other candidate allelochemicals (Kato *et al* 2002; Sondhia and Saxena 2003, 2004) [25, 26]. In spite of strong herbicidal activity of *X. strumarium* crude saponin on *V. sativa* seedlings, much multidisciplinary research is required. The fact that saponin is a natural product with potent herbicidal activity makes this topic more worthy of future investigation. Thus, pentacyclic saponin molecule isolated from *X. strumarium* may serve as a potential herbicide template in developing of new molecule for controlling weeds in future.

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