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In vitro Antifungal Activities of Extracts of Fruits and other Morphological Parts of *Xanthium strumarium* Against the Plant Pathogen, *Rhizoctonia solani*

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Abstract. *In vitro* antifungal activity of different plant parts of *Xanthium strumarium* (Compositae) was investigated against *Rhizoctonia solani* to seek safe natural alternatives to the harmful synthetic fungicides. The most active plant parts of *X. strumarium* were seeds, extracted with *n*-hexane and the leaves, extracted with absolute ethanol. The two treatments resulted in growth inhibition diameters of 45 mm and 47 mm, respectively. The value of MIC lied between 350.0 and 175.0 µg of *Xanthium* oil/mL. Gas liquid chromatography of the seed oil of *X. strumarium* revealed the presence of the usual fatty acids, palmitoleic (7.6%), oleic (21.6%) and linoleic (70.4%). The oil was separated into free fatty acids fraction and unsaponifiable matter fraction. The unsaponifiable matter fraction was separated on TLC, out of six separated compounds, two were active against *R. solani.* The infrared spectra (FTIR) of these two purified compounds pointed to a long chain hydrocarbon back-bone for both, one of them possessing in addition, an alcoholic moiety.

Keywords: *Xanthium strumarium*, antifungal activity, *Rhizoctonia solani*, seed-oil, unsaponifiable matter fraction

Introduction

Rhizoctonia solani is a serious soil and seed borne pathogen that causes damping off disease resulting in severe losses all around the world, to various crop plants including important food crops (Kataria and Verma, 1992; Anderson, 1982) as well as forest trees (Camporota and Perrin, 1998). The fungus was first reported on wheat in Sudan (Elnur and Chester, 1967). We have also observed the fungus to infect other crops, e.g., cotton, sorghum, tomato and onions.

R. solani is very persistent in soil and only very few chemical fungicides are effective against it (Errampali and Johnson, 2001). Other, non-chemical methods are attempted for control of the fungus and most notably used is biological control agents (Fiddaman and Rossall, 1995).

Natural chemicals of plant origin represent a diversified, renewable and a safe source of potential pesticides. Natural antifungal products of plant origin were reviewed by Arif *et al*. (2009). *Xanthium strumarium*, commonly known as cocklebur or bur weed, belongs to the family Compositae and is an annual gregarious weed widely distributed in many parts of the world (Favier *et al*., 2005). Cocklebur enjoys a reputation in the folk medicine of several nations of the world as a remedy for a number of ailments including malaria, rheumatism, leprosy, etc. (Kamboj and Saluja, 2010). In addition to being toxic to man and animals, cocklebur was reported to possess interesting biological or pharmacological activities including antitrypanosomal (Talakal *et al*., 1995), anti-cancer (Ramirez-Erasa *et al*., 2007), anti-inflammatory (Han *et al*., 2007) and other activities (Akarte *et al*., 2009; Yoon *et al*., 2008).

X. strumarium is an invasive widely spreading weed in Sudan particularly in the irrigated agricultural schemes in central Sudan. The spiny fruits easily cling to the hair of grazing animals, aiding seed dissemination as well as causing some animal health problems. The rainy season, in addition, supports considerable growth of the plant outside these schemes, as part of the spontaneous autumn (Kharif) flora. Thus the plant's biomass is available in quantity. Crude extracts of *X. strumarium* have been shown to possess antifungal activity, although against fungi other than *R. solani* (Park *et al*., 2005).

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The objectives of this work was to test solvent extracts prepared from different morphological parts of *X. strumarium* for their activity against the plant disease causing fungus *R. solani* and to chemically characterise the active antifungal ingredients.

Meterials and Methods

Collection and preparation of plant material. Samples of wild-growing *X. strumarium* plants were collected from the Blue Nile river banks near the Gezira University area at Wad Medani city, Sudan (Herbarium No. NOPRI. NEOUprjt.005). The collected plants were dissected into morphological parts (leaves, roots, etc.) and allowed to dry at ambient temperature in the shade before grinding and subsequent solvent extraction.

Solvents and chemicals. All solvents, chemicals and media used in bioassays were obtained from E. Merck (Germany) or Sigma (USA).

Preparation of solvent extracts from *Xanthium* plant parts. Air-dried, powdered *Xanthium* plant material was extracted using either of three methods. The first was a cold method that involved maceration of powdered tissue with 20 volumes of solvent such as ethanol (95%) or *n*-hexane using conical flasks maintained at room temperature for 72 h with mild magnetic stirring. In addition two hot extraction methods were (a) involving Soxhlet extraction and (b) refluxing in an appropriate solvent. All extracts and their filtrates were concentrated in a rotatory evaporator and kept for further chemical analyses and bioassay.

***Xanthium* seed-oil fractionation.** *Xanthium* seed-oil was partially saponified using KOH, according to AOCS method (1993), and fractionated into free fatty acids and the unsaponifiable matter fractions. After saponification the unsaponifiable matter was recovered in chloroform. The remaining saponified fraction was acidified and free fatty acids were extracted with hexane.

Preparative thin layer chromatographic separations. The unsaponifiable matter fraction prepared as above from 1g of *Xanthium* seed oil was dissolved in a final volume of 10 mL of chloroform. Volumes of 100 µL of this solution were each applied to a TLC plate (20× 20 cm glass plates pre-coated with silica gel 60, without fluorescent indicator, layer thickness 0.5 mm; Merck, Germany). The plates were developed in a tank containing the solvent mixture, *n*-hexane: acetone (4:1). Bands were detected using the Antimony trichloride reagent. Provisions were made to detect separated components only at the edge of the plate, allowing recovery of others (to the inside of the plate), uncontaminated by detection reagent. These bands were scraped individually, transferred to sterilised sample bottles, eluted with solvent and taken for further studies.

Transmethylation of *Xanthium* oil fatty acids. Fatty acid methyl esters were derivatised using 0.5 N sodium methoxide. About 0.5 g of the oil was weighed in a stoppered flask and 0.5 N sodium methoxide was added (7-8 mL). The contents were heated for 3-5 min in a water-bath at 80 °C with shaking and then transferred to a separatory funnel. A few drops of glacial acetic acid were added followed by 15 mL of distilled water and 10 mL of *n*-hexane. After equilibration, the *n*-hexane layer was collected and kept dry over anhydrous sodium sulphate before GLC analysis.

Gas liquid chromatography. The fatty acid composition was determined by GLC using a Varian 3400 gas liquid chromatograph equipped with a hydrogen flame ionisation detector and a computing integrator. A fused silica gel column (25 m × 0.32 mm inner diameter) packed with polyethylene glycol-2-nitroterephthalic ester (film thickness 0.25 µm) was used. Analysis of fatty acid methyl esters was carried out isothermally at column oven temperature of 170 °C, column inlet and detector oven temperature of 180 °C and the carrier gas flow was 50 mL/min. The identification of fatty acids was carried out according to the retention time of esters of authentic samples of fatty acid methyl esters.

Bioassay of antifungal activity. Isolated and identified *Rhizoctonia solani* fungus was grown on PDA medium. Micro fibre glass discs (5 mm in diameter) saturated with plant extracts, as well as control solutions, were placed in the middle of the plate for 24 h before culturing (using the disc diffusion method essentially described by Bauer *et al*., 1966). Two discs (5 mm in dia) of the test fungus culture, were prepared using a cork borer, placed from each side of the petri-plates. Micro fibre discs saturated with the respective solvent, alone, were used as control treatments. The growth diameter (three replicas for each test) was periodically measured for up to three days and the inhibition zone was expressed in millimetre (± Standard Deviation).

Results and Discussion

Table 1 shows fungal inhibitory effects on the growth of *R. solani* Kuhn of crude extracts prepared from different morphological parts (seed, stem, leaf and root)

Table 1. Fungal growth inhibitory activities against *R. solani*, of crude extracts prepared from different parts of *X. strumarium* using different extraction methods and solvents. The disc diffusion method was used for antifungal assays

Treatment no.	Extraction		Zones of fungal growth inhibition (mm)			
	Solvent	Method	Seed	Stem	Leaf	Root
1.	Ethanol, absolute	Soxhlet	0.0	12.1 ± 0.2	37.2 ± 0.3	40.3 ± 0.1
2.*	Ethanol, absolute	Soxhlet	10.4 ± 0.2	-	-	-
3.	Ethanol, absolute	Maceration	-	-	38.9 ± 0.3	38.0 ± 0.4
4.*	Ethanol, absolute	Maceration	-	-	47.5 ± 0.4	45.1 ± 0.4
5.	Ethanol, absolute	Reflux	-	-	34.5 ± 0.3	35.0 ± 0.3
6.	Ethanol, aqu. 70%	Maceration	-	-	2.5 ± 0.3	30.3 ± 0.4
7.	*n*-Hexane	Soxhlet	32.0 ± 1.0	-	0.0	0.0
8.	Dichloromethane	Maceration	-	-	40.2 ± 0.2	40.6 ± 0.3

Notes: All pure solvent controls gave a fungal inhibition zone of 0.0 mm; (*) in treatments 2 and 4, plant material was first defatted by hexane extraction (Soxhlet) before preparing the absolute ethanol extract; (-) = not determined; aqu. = aqueous.

of cocklebur (*X. strumarium* L.). The dry powdered plant material was directly extracted with solvent, however, in two occasions (indicated in the Table) the plant material was first defatted by Soxhlet-extraction with hexane followed by re-extraction of the marc (Soxhlet) with absolute ethanol; the latter extract assessed for antifungal activity. Solvents used were absolute ethanol, 70% aqueous ethanol, *n*-hexane and dichloromethane.

The most active absolute ethanol extracts (Soxhlet method) were those of the plant roots and leaves, resulting in fungal growth inhibition diameters of 40 and 37 mm, respectively (Table 1, Treatment no.1). The ethanol extract of the stems was less active, while, that of the directly extracted seeds was completely inactive against *R. solani*. However, pre-extraction of the seeds with hexane (defatting) followed by subsequent extraction of the marc with absolute ethanol, resulted in a considerably active ethanol extract (Table 1, Treatment no. 2). On the other hand, hexane extracts of the seeds prepared by the Soxhlet method (consisting of the seed fixed oil) were very active, resulting in a fungal growth inhibition zone of 32.0 mm (Fig. 1), while, hexane extracts of the leaves and roots were completely inactive towards growth of *R. solani*.

A comparison of the efficiency of the three extraction methods (Soxhlet, maceration and reflux methods) in recovering the antifungal constituent(s) from *Xanthium* tissue can be made by considering Table 1 (Treatments no. 1, 3 and 5) in which absolute ethanol was used to extract leaf and root morphological parts. The three methods gave similar results of recovery of antifungal activity, although the reflux method yielded somewhat lower activity.

Using the maceration method, dichloromethane, as extraction solvent was as good as or slightly better than absolute ethanol in the cases of both leaf and root plant material (Table 1, Treatments no. 3 and 8). However, aqueous 70% ethanol gave an extract with less inhibitory activity towards the growth of *R. solani* especially when used with the leaf material (Table 1, Treatments no. 3 and 6). Again, as observed for seed material, prior defatting (extraction with hexane) followed by extracting the marc with absolute ethanol resulted in increased activity of absolute ethanol extracts of both leaves and roots (Table 1, Treatments no. 3 and 4).

Bahraminejad *et al*. (2011) screened 63 Iranian plant species, including *X. strumarium*, against three phytopathogenic fungi, including *R. solani*. These authors

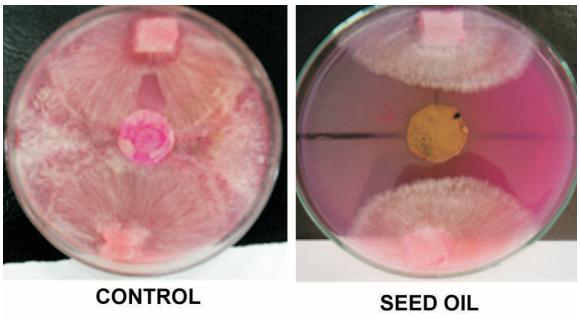

Fig. 1. Typical photos of inhibition of the growth of *R. solani* by the seed oil (hexane extract) of *Xanthium*.

reported weak activity for aqueous extracts of the shoots of *X. strumarium*, but considerable activity for the plant's shoot methanol extracts. So far, this is the only report available on the antifungal effect of extracts of *X. strumarium* on *R. solani*. Crude extracts of cocklebur (*X. strumarium*) have been reported to possess considerable antifungal activity against fungal species of *Phytophthora* (Bahraminejad, 2012; Kim *et al*., 2002), *Sclerotinia* and *Aspergillus* (Park *et al*., 2005). On the other hand, Murillo-Alvarez *et al*. (2001) reported a low activity for ethanol extracts of *X. strumarium* tested among extracts of 24 other plants, against *Candida albicans*. Gupta and Banerjee (1972) also tested crude extracts of 170 different West Bengal plants against the two fungi *Aspergillus niger* and *Trichophyton rubrum* and reported no antifungal activity for aqueous or ethanolic extracts of leaf, stem, root or fruit of *X. strumarium*. Extracts of other species of *Xanthium* also showed antifungal activity against fungi not including *R. solani*, e.g., *X. spinsum* (Ginesta-Peris *et al*., 1994) and *X. macrocarpum* (Lavault *et al*., 2005). Present report is the first to deal with growth inhibitory effects against *R. solani*, of extracts of any species of *Xanthium* prepared by different methods from morphological parts of the plant.

There are, however, reports of preparations made from plant species not belonging to the genus *Xanthium* that showed growth inhibition of *R. solani.* These included the volatile oil of *Chenopodium ambrosioides* (Dubey *et al*., 1983), dichloromethane extracts of *Desmos chinensis* (Plodpai *et al*., 2013) and a preparation obtained by mechanical expression of *Aloe vera* (Rodriguez *et al*., 2005).

It is important to choose the right extraction solvent properly for different morphological parts of the plant. The fact that antifungal activity of the seeds of *X. strumarium* was only when soluble in hexane (and not in absolute ethanol) and the reverse solubility property for compound(s) responsible for antifungal activity of the leaf and root (Table 1, Treatments no. 1 and 7) suggests the presence of at least two different types of compounds active against *R. solani*.

Improved recovery of antifungal constituents by absolute ethanol extraction, following removal of hydrophobic constituents (defatting) of *Xanthium* seed, leaf and root material (Table 1, Treatment nos. 1, 2 and 3, 4) suggests that some antifungal *Xanthium* constituent(s) has a distinct hydrophobic moiety.

Stability of antifungal activity of crude cocklebur extracts. Three extracts of *X. strumarium* were prepared from the seeds (using the Soxhlet method and hexane as extraction solvent) as well as from the leaves and roots of the plant (using the maceration method and absolute ethanol as solvent). Aliquots from each of the three extracts were applied to 5 mm diameter discs in amounts corresponding to equivalent plant powder weight of each plant part. The discs were then placed on the growth media used for the usual antifungal bioassay protocol and allowed to stand for time periods of 0, 24, 48, 72 and 96 h (at room temperature), before the antifungal bioassay was started by adding cultures of *R. solani*. Thus these time periods represent different times of storage of *Xanthium* extracts at room temperature. The results are shown in Table 2. With all three *Xanthium* extracts, fungal growth inhibitory activity somewhat increased initially with storage and was maximal in extract-loaded discs stored for 48 h before the fungus was introduced (Table 2). This increase could be due to increased diffusion of the antifungal components of the extracts with incubation time. Thereafter, antifungal activity only slightly decreased. After 96 h (4 days) of storage of the discs at room temperature, 100, 86 and 75% of the original antifungal activities of all three extracts still remained. Thus, the three extracts, particularly those of the seed were quite stable under the conditions of the test.

Table 2. Stability of fungal inhibitory activity (against R. solani) of Xanthium seed, leaf and root extracts stored at room temperature for different time periods

Storage time	Fungal inhibition zone (mm)		
(h)	Seed	Leaf	Root
0	30.0	40.0	37.0
24	34.0	35.0	40.0
48	45.0	45.0	47.0
72	43.0	42.0	45.0
96	30.0	30.0	30.0

The values represent means of three separate bioassay determinations.

Minimum inhibitory concentration (MIC) of the seed oil of cocklebur towards *R. solani*. Figure 2 shows antifungal activity against *R. solani* of different concentrations of *X. strumarium* seed oil obtained by Soxhlet extraction with *n*-hexane. The inhibition zone of fungal growth decreased with decreasing *Xanthium* seed oil concentration used to saturate the assay discs. The value of MIC lied between 350.0 and 175.0 μg of *Xanthium* seed oil/mL.

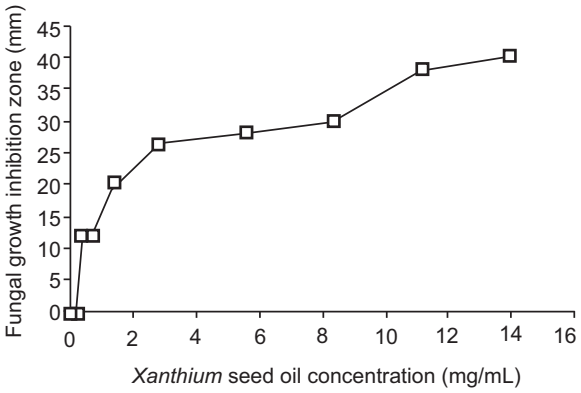

Fig. 2. Inhibition of *R.solani* growth by different concentrations of *Xanthium* seed oil.

Partial characterization of the active antifungal ingredients of cocklebur seed oil. The seed oil was chosen for further studies since it was quite active against *R. solani* and represented a somewhat more homogenous (lipid) fraction.

It is known that many of the so-called 'unusual' fatty acids, such as short-chain, cyclopropanoid or acetylenic fatty acids have antifungal activity (Carballeira, 2008). However, GLC analysis (Table 3) revealed that the oil contained no unusual fatty acids. Linoleic and oleic acids constituted over 90% of cocklebur seed oil fatty acids. Palmitoleic acid, a usual fatty acid was present in a relatively larger amount. Cocklebur seed oil was further separated into two fractions, the free fatty acid (FFA) and the unsaponifiable matter (UM) fractions. Both fractions were assayed for their inhibition of the growth of *R. solani*. No antifungal activity was detected for the FFA fraction and it was associated solely with the UM fraction (Table 4, Fig. 3). Subsequently the UM fraction was subjected to preparative TLC analysis yielding six major components. These were numbered (I) to (VI), according to increasing R_f value in the TLC solvent system *n*-hexane: acetone (4:1). Bands representing each of these components were scraped, eluted in a solvent and assayed for growth inhibition of *R. solani*. Two of the six TLC spots, designated as II and IV were active against the growth of *R. solani* (Table 5). The two compounds were subjected to infrared spectroscopic analysis. Thus, it is concluded that compound II is a long chain alcohol (absorption at 3453 cm^{-1} due to O-H; at 1165 cm^{-1} due to C-O; relatively intense absorption at 2960-2850 cm^{-1} and long chain methyl rocking at 725 cm^{-1}). The presence of a hydrophobic moiety (the long hydrocarbon chain) is consistent with the results observed for increased recovery of antifungal activity from seed, leaf and root tissues of *Xanthium* on defatting. This feature may assume significance in future studies on the mode of action of this antifungal compound.

Table 3. Fatty acid composition of *Xanthium* seed oil as determined by GLC

Fatty acid	%
Palmitic acid	0.5
Palmitoleic acid	7.2
Stearic acid	0.7
Oleic acid	21.6
Linoleic acid	70.0

Table 4. Antifungal activity of the two oil fractions of *X. strumarium* on growth of *R. solani*

Oil fraction	Fungal growth inhibition (mm)
Free fatty acids	0.0
Unsaponifiable matter	28.0

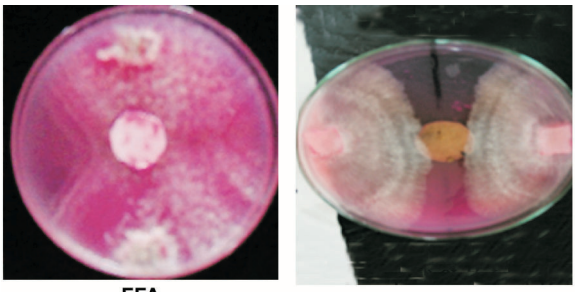

Fig. 3. Inhibition of the growth of *R. solani* by the free fatty acid (FFA) and unsaponifiable matter (UM) fractions of *Xanthium* seed oil.

Table 5. Antifungal activity of TLC separated components of *Xanthium* seed oil unsaponifiable fraction

Components	Fungal growth inhibition zone (mm)
I	0.0
II	30.0
III	0.0
IV	20.0
V	0.0
VI	0.0
Control*	0.0

* = eluate of blank (spot-free) silica gel.

References

Akarte, A.S., Bhagat, V.C., Deshmukh, P.T., Disle, C. 2009. Evaluation of antinociceptive effect of *Xanthium strumarium* Linn. leaves extract in Swiss albino mice. *International Journal of Green Pharmacy*, **3:** 151-154.

Anderson, N.A. 1982. The genetics and pathology of *Rhizoctonia solani*. *Annual Review of Phytopathology*, **20:** 329-347.

AOCS, 1993. *The Official Methods and Recommended Practices of the American Oil Chemists Society*, D. Firestone, (ed.), 4th edition, AOCS Press, Champaign, Illinois, USA.

Arif, T., Bhosale, J.D., Kumar, N., Mandal, T.K., Bendre, R.S., Lavekar, G.S., Dabur, R. 2009. Natural products- antifungal agents derived from plants. *Journal of Asian Natural Products Research*, **11:** 621-638.

Bahraminejad, S., Abbasi, S., Maassoumi, S.M., Tebein, S. 2012. Evaluation of inhibitory effects of extracts of plants from western Iran against *Phytophthora drechsleri*. *Australian Journal of Crop Science*, **6:** 255-260.

Bahraminejad, S., Abbasi, S., Fazalali, M. 2011. *In vitro* antifungal activity of 63 Iranian plant species against three different plant pathogenic fungi. *African Journal of Biotechnology*, **10:** 16193-16201.

Bauer, R.W., Kirby, M.D.K., Sherris, J.C., Truck, M. 1966. Antibiotic susceptibility testing by standard single disc diffusion method. *American Journal of Clinical Pathology*, **45:** 493-496.

Camporota, P., Perrin, R. 1998. Characterization of *Rhizoctonia* species involved in tree seedling damping-off in French forest nurseries. *Applied Soil Ecology*, **10:** 65-71.

Carballeira, N.M. 2008. New advances in fatty acids as antimalarial, antimycobacterial and antifungal agents. *Progress in Lipid Research*, **47:** 50-61.

Dubey, N.K., Kishore, N., Srivastava, O.P., Dikshit, A., Singh, S.K. 1983. Fungitoxicity of some higher plants against *Rhizoctonia solani*. *Plant and Soil*, **72:** 91-94.

Elnur, E., Chesters, C.G.C. 1967. A note on two isolates of *Rhizoctonia solani* Kuhn from wheat. *Plant Pathology*, **16:** 104-107.

Errampalli, D., Johnston, H.W. 2001. Control of tuber-borne black scurf (*Rhizoctonia solani*) and common scab (*Streptomyces scabies*) of potatoes with a combination of sodium hypochlorite and thiophanatemethyl preplanting seed tuber treatment. *Canadian Journal of Plant Pathology*, **23:** 68-77.

Favier, L.S., Maria, A.O.M., Wendel, G.H., Borkowski, E.J., Giordano, O.S., Pelzer, L., Tonn, C.E. 2005. Anti-ulcerogenic activity of xanthanolide sesquiterpenes from *Xanthium cavanillesii* in rats. *Journal of Ethnopharmacology*, **100:** 260-267.

Fiddaman, P.J., Rossall, S. 1995. Selection of bacterial antagonists for the biological control of *Rhizoctonia solani* in oilseed rape (*Brassica napus*). *Plant Pathology*, **44:** 695-703.

Han, T, L., H.-L., Zhang, Q.-Y., Han, P., Zheng, H.-C., Rahman, K., Qin, L.-P. 2007. Bioactivity-guided fractionation for anti-inflammatory and analgesic properties and constituents of *Xanthium strumarium* L. *Phytomedicine*, **14:** 825-829.

Ginesta-Peris, E., Garcia-Breijo, F.J., Primo-Yufera, E. 1994. Antimicrobial activity of xanthatin from *Xanthium spinosum* L. *Letters in Applied Microbiology*, **18:** 206-208.

Gupta, S.K., Banerjee, A.B. 1972. Screening of selected West Bengal plants for antifungal activity. *Economic Botany*, **26:** 255-259.

Kamboj, A., Saluja, A.K. 2010. Phytopharmacological review of *Xanthium strumarium* L. (Cocklebur). *International Journal of Green Pharmacy*, **4:** 129-139.

Kataria, H.R., Verma, P.R. 1992. *Rhizoctonia solani* damping-off and root rot in oilseed rape and canola. *Crop Protection*, **11:** 8-13.

Kim, D.K., Shim, C.K., Bae, D.W., Kawk, Y.S., Yang, M.-S., Kim, H.K. 2002. Identification and biological characteristics of an antifungal compound extracted from cocklebur (*Xathium strumarium*) against *Phytophthora drechsleri*. *Plant Pathology Journal*, **18:** 288-292.

Lavault, M., Landreau, A., Larcher, G., Bouchara, J.P., Pagniez, F., LePape, P., Richomme, P. 2005. Anti-leishmanial and antifungal activities of xanthanolides isolated from *Xanthium macrocarpum*. *Fitoterapia*, **76:** 363-366.

Murillo-Alvarez, J.I., Encarnacion, D.R., Franzblau, S.G. 2001. Antimicrobial and cytotoxic activity of some medicinal plants from Baja California Sur (Mexico). *Pharmaceutical Biology*, **39:** 445-449.

Park, S.-M., Jung, H.-J., Han, S.-H., Yeo, S.-H.W., Kim, Y.-W., Ahn, H.-G., Kim, H.-S., Yu, T.-S. 2005.

Antifungal activity of extract from *Xanthium strumarium* L. against plant pathogenous fungi. *Journal of Life Science*, **15:** 692-695.

Plodpai, P., Chuenchitt, S., Petcharat, V., Chakhong, S., Voravuthikunchai, S.P. 2013. Anti-Rhizoctonia solani activity by Desmos chinensis extracts and its mechanism of action. *Crop Protection*, **43:** 65-71.

Ramirez-Erosa, I., Huang, Y., Hickie, R.A., Sutherland, R.G., Barl, B. 2007. Xanthatin and xanthinosin from the burs of *Xanthium strumarium* L. as potential anticancer agents. *Canadian Journal of Physiology and Pharmacology*, **85:** 1160-1172.

Rodriguez, D.J., Hernandez-Castillo, D., Rodriguez-Garcia, R., Angulo-Sanchez, J.L. 2005. Antifungal activity *in vitro* of *Aloe vera* pulp and liquid fraction against plant pathogenic fungi. *Industrial Crops and Products*, **21:** 81-87.

Talakal, T.S., Dwivedi, S.K., Sharma, S.R. 1995. In vitro and *in vivo* antitrypanosomal activity of *Xanthium strumarium* leaves. *Journal of Ethnopharmacology*, **49:** 141-145.

Yoon, J.H., Lim, H.J., Lee, H.J., Kim, H.-D., Jeon, R., Ryu, J.-H. 2008. Inhibition of lipopolysaccharide-induced inducble nitric oxide synthase and cyclooxygenase-2 expression by xanthanolides isolated from *Xanthium strumarium*. *Bioorganic and Medicinal Chemistry Letters*, **18:** 2179-2182.