



Phytochemical and cytogenetic studies of medicinally important *Oxalis* species occurring in India: A critical review

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ABSTRACT

The paper is a comprehensive review of the work done on cytogenetic and phytochemical evaluation of three medicinally important species of *Oxalis* of India. Compared to the other two, *Oxalis corniculata* is a well-studied species with regard to its phyto-constituents, antioxidant and antimicrobial properties. While *O. corniculata* and *O. debilis* are used in traditional system of medicine, *Oxalis triangularis* is grown as an ornamental plant in gardens. In view of the folk medicinal uses, *O. debilis* is attracting attention of workers in recent times and publications on phyto-constituents, pharmaceutical, toxicological, anti-microbial and nutraceutical properties have been brought out. The current review highlights the medicinal importance of *O. debilis* and *O. triangularis* and emphasizes the need for future research in the field of phytochemistry, pharmaceutical evaluation, bioactivity studies, cytogenetic and molecular studies for optimization of drug yield and genetic improvement of these two species of *Oxalis*.

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1. Introduction

Medicinally active compounds are the natural products of plant metabolites and discovered since thousands of years ago. Traditional medicine plays an important role in human health and is used by over 80% of the world's population for primary healthcare (Fainsworth, 1985) as they are cost effective and have not much side effects (Agarwal *et al.*, 2011). Ayurvedic and Unani medicines are also make use the benefits of herbal drugs. About 1000 B.C., Charaka Samhita has documented the use of over 2000 herbs of medicinal value, which have provided us some important lifesaving drugs (Goyal *et al.*, 2007). Herbal drugs are safer to the human body compared to synthetic drugs. Hence, different laboratories are engaged in screening of plants for biologically active therapeutics and potential compounds. Thus, investigations on chemical constituents of the plants are important in developing medicinally important novel molecules of high therapeutic values. Importance of dietary items as nutraceutical elements has been realized and their significance in the treatment of chronic diseases has been

highlighted (Williamson *et al.*, 1996).

The genus *Oxalis* of the family Oxalidaceae is comprised of about 570 species and is distributed in America, Africa, Asia and Europe (Christenhusz & Byng, 2016; Loutieg, 2000). This genus contributes to about 89% of the total species diversity of the family. The members of *Oxalis* are morphologically variable, which includes shrubs, herbs, stem succulents, annuals and geophytes, with a cosmopolitan distribution. In India, the genus *Oxalis* L. is represented by 10 species (Manna, 1997) with larger species concentration in Kashmir (Muzafar *et al.*, 2015). Three species of *Oxalis* namely, *Oxalis corniculata*, *O. debilis* and *O. triangularis* are widely distributed in Indian sub continent (Figs. 1a-1c). *Oxalis debilis* Kunth is a cosmopolitan, gregarious and perennial herb having long petiole and is believed to be native to South America. At present, it has spread and naturalized in several tropical countries including Hawaii, Fiji, New Caledonia, Australia and the Galapagos Islands (Lourteig, 2000). The present review is a comprehensive account of the past work done on cytogenetic and

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Fig. 1: Habit and flowering plants of (A) *Oxalis corniculata* (B) *O. debilis* and (C) *O. triangularis*

phytochemical evaluation of three medicinally important species of *Oxalis* (*O. corniculata*, *O. debilis* and *O. triangularis*) occurring in India.

Oxalis corniculata L., commonly known as “Creeping Wood Sorrel” is wide spread in India with several traditional medicinal uses. The plant is a delicate and low-growing herb characteristic of shady and moist localities, lawns, roadsides and forest floors in warmer part of country. *Oxalis debilis*, commonly known as “Large-flowered Pink Sorrel” or “Pink Wood Sorrel” is native to South America (Denton, 1973; Lourteig, 2000) but has become a very cosmopolitan species in all continents except Antarctica. It has got naturalized in different subtropical and tropical parts of India especially in the Brahmaputra Valley region of the North Eastern part of India (Junejo *et al.*, 2016) and Kashmir (Muzafar *et al.*, 2015). *Oxalis debilis* has uniform distribution in Assam including Dibrugarh district and is locally called as Bor-tenggshi (Assamese) and is an important wild edible plant which is often incorporated in various traditional cuisines like sour fish and bottle gourd dishes (Patiri and Borah, 2012). The species has traditional medicinal use in various major health complications like in treatment of diarrhea, diabetes, piles and scurvy besides its use as an antidote for toxicity (Singh and Dubey, 2012) in different parts of the India. This perennial herb flowers from March to September and being a tristylous species without seeds, it mainly propagates through bulbils. The tiny bulbils have a prodigious ability to persist for several years in soil, which can germinate on attaining favorable conditions (Luo *et al.*, 2006). It is used for treatment of various ailments such as diarrhea by local people (Kirtikar and Basu, 1988), but there was no report on the antioxidant activity and nutraceutical properties of *O. debilis* until the publication by Sarma *et al.* (2015) was brought out. Two taxonomic varieties such as *O. debilis* var. *debilis* and *O. debilis* var. *corymbosa* are recognized within the species. The basic difference between the two varieties of *O. debilis* is the manner in which the

clusters of oxalate crystals are arranged; whether they are along the leaf margin or spaced evenly throughout the lamina. In South America, the range of distribution of the two varieties overlap and both set seeds. Besides, the two varieties are also frequently cultivated as ornamental plants and have become naturalized throughout the world (Lourteig, 2000).

Oxalis triangularis A. St-Hil., known as “False Shamrock”, is endemic to Brazil and has attracted worldwide due to its fish tail like leaf shape and beautiful pinkish-purple colour. This woodsorrel is a perennial, typically grown as a houseplant but can be grown outside, preferably in light shade. The plant is edible and mostly propagates through their bulbils (Taha *et al.*, 2013). The current review deals with work done on the medicinal properties, phytochemical diversity and cytogenetics of three species of *Oxalis* viz. *Oxalis corniculata*, *O. debilis* and *O. triangularis*, growing in the wild or as cultivated plants in India.

2. Phytochemical characteristics

There are several published reports on the phytochemical constituents of *O. corniculata*. In contrast, very scanty information is available of the phytoconstituents of *O. debilis* and *O. triangularis*. Our work on TLC and HPTLC profiling of methanolic extracts of *O. debilis* has shown promising result that revealed the presence of a number of phytochemicals. TLC analysis of methanolic extract of four ecotypes of *O. debilis* in one solvent system indicated the presence of diverse type of phytochemicals in this plant. In methanolic extracts of *O. debilis*, HPTLC fingerprint established the qualitative and quantitative presence of phytoconstituents (Figs. 2 and 3A-D). HPTLC chromatograms of different ecotypes of *O. corniculata* and *O. debilis* showed qualitative and quantitative variation in phytocompounds. The reported phytochemical constituents in three species of *Oxalis* under review in presented in Table 1. Among ecotypes of each plant samples, methanolic extracts of *O. debilis* possessed phytocompounds with higher

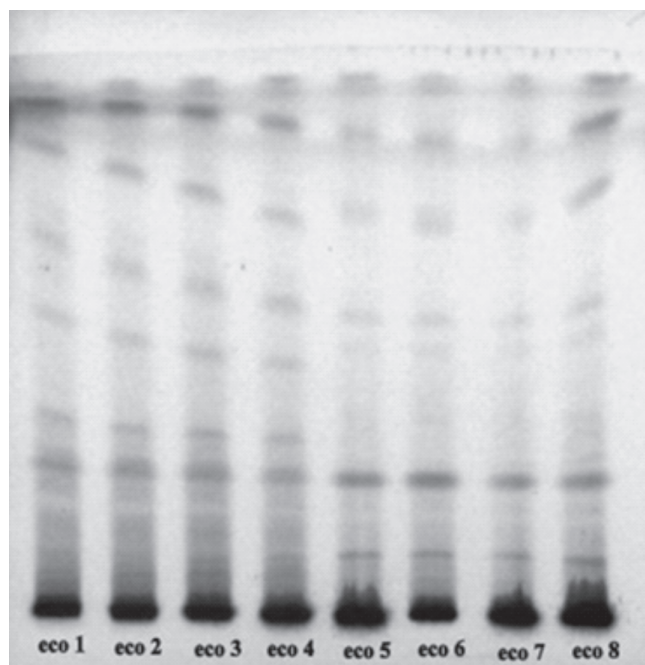


Fig. 2: TLC plate showing various bands in methanolic extracts of ecotypes of *O. corniculata* (eco-1, eco-2, eco-3, and eco-4) and *O. debilis* (eco-5, eco-6, eco-7, eco-8) under UV light 254 nm

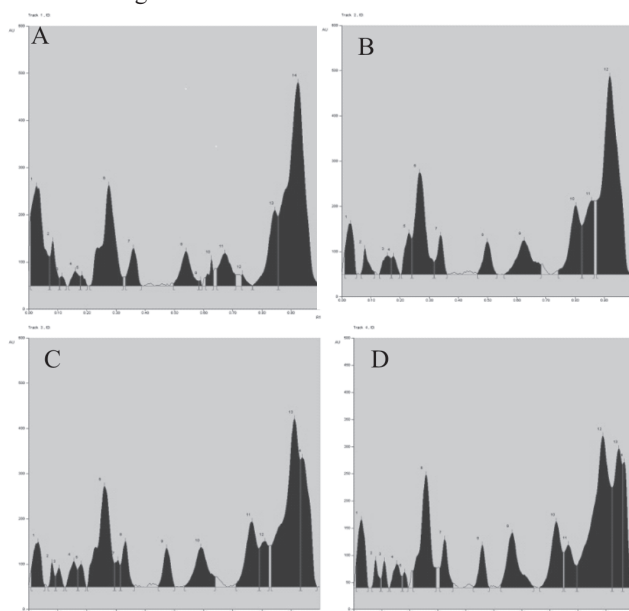


Fig. 3: HPTLC chromatograms of different ecotypes of methanolic extracts of *O. corniculata* (A=eco-1, B=eco-2, C=eco-3, D=eco-4) showing various peaks.

number of peaks. HPTLC chromatograms of each ecotype showed little variation in Rf values (Figs. 2-4). Area occupied by the phytochemicals and their peaks also differed considerably as earlier reported by us (Panda *et al.*, 2016).

3. Nutraceutical properties

The leaves of *O. corniculata* are tangy in taste, rich in moisture, crude carbohydrate, protein and fibre and therefore,

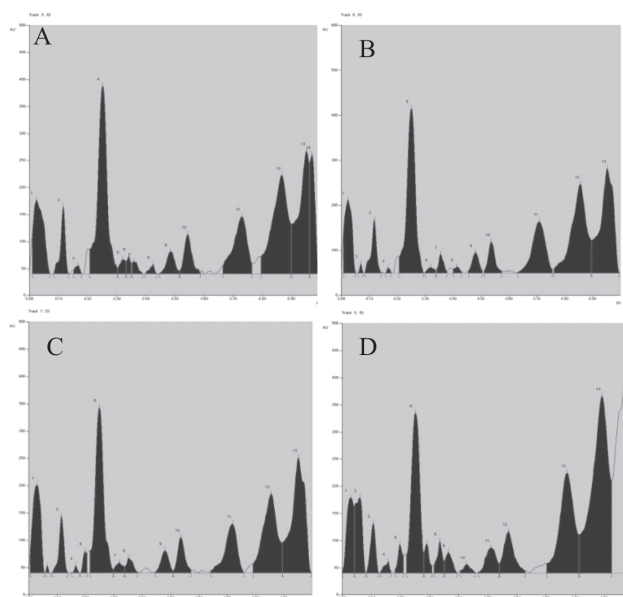


Fig. 4: HPTLC chromatograms of different ecotypes of methanolic extracts of *O. debilis* (A=eco-1, B=eco-2, C=eco-3, D=eco-4) showing various peaks.

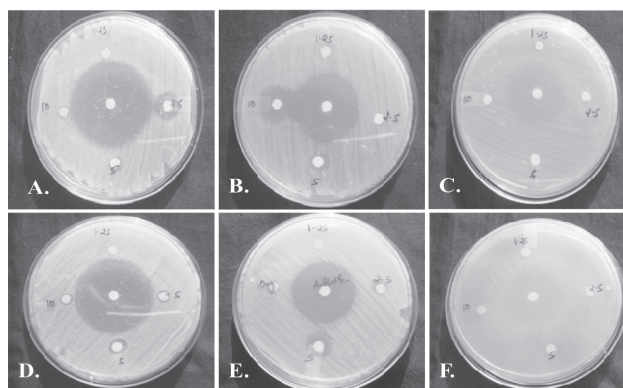
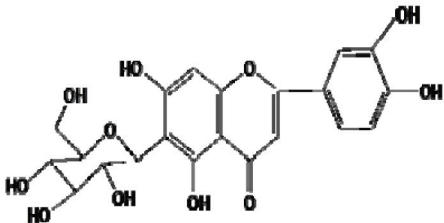
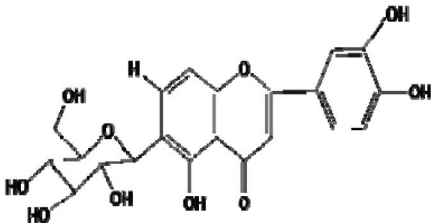
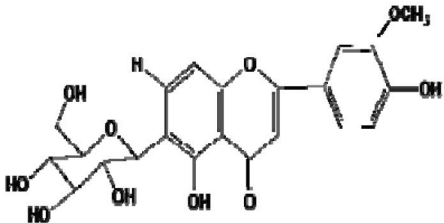
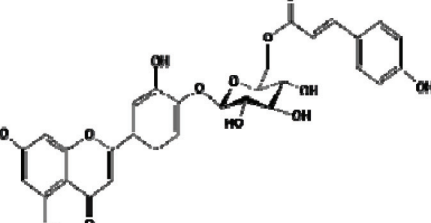
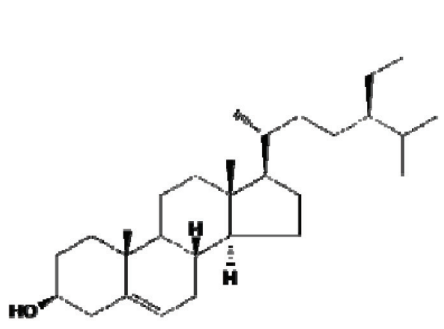
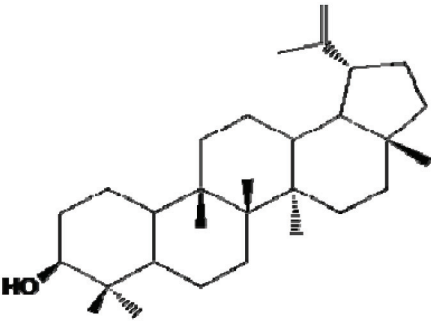


Fig. 5: (A-C): Antibacterial activity by methanolic extract of *O. corniculata* against (A) *Bacillus subtilis* (B) *Streptomyces epidermidis* and (C) *Pseudomonas aeruginosa*. Fig. 5 (D-F): Antibacterial activity by methanolic extract of *O. debilis* against (D) *Bacillus subtilis* (E) *Streptomyces epidermidis* and (F) *Pseudomonas aeruginosa*

could be considered as an alternative to vegetable in case of emergency or food scarcity. The leaves contain sodium (1.12%), calcium (2.5%) and nitrogen (3.5%) as reported by Ibrahim (2012). Moisture content of the powdered leaf of *O. debilis* has been found to be ~11.09% (Junejo *et al.*, 2016). The carbohydrate content was higher in *O. debilis* (6.40 $\mu\text{g}/\text{mg}$) than that of *O. corniculata* (3.81 $\mu\text{g}/\text{mg}$). The protein content of *O. corniculata* and *O. debilis* was quite comparable in both the species and was found to be 0.29 $\mu\text{g}/\text{mg}$ and 0.31 $\mu\text{g}/\text{mg}$ respectively. The crude fibre content was calculated as 65.5% and 62.00% in *O. corniculata* and *O. debilis* respectively. Traditionally, *O. debilis* is used for treatment of various ailments such as diarrhea (Sarma *et al.*, 2015) but the medicinal use of *O. triangularis* is not known.

Table 1

Phytochemical constituents of different species of *Oxalis*

Chemical structure of reported phytoconstituents	References	
<i>Oxalis corniculata</i>		
<p data-bbox="164 421 1117 521">Flinolenic acid, stearic acid, phenolic acids like p- hydroxybenzoic, vanillic and syringic acid, flavonoids like isoorientin, isovitexin and swertisin, flavons like acacetin and 7,4' – diOMe orientin, flavonols (3', 4'- di OMe quercetin), Corniculatin A.</p> <div style="display: flex; justify-content: space-around;"> <div data-bbox="183 559 626 783">  <p data-bbox="175 817 318 846">Iso-orientin</p> </div> <div data-bbox="659 559 1089 783">  <p data-bbox="651 817 794 846">Isovitexin</p> </div> </div> <div style="display: flex; justify-content: space-around; margin-top: 20px;"> <div data-bbox="183 889 626 1112">  <p data-bbox="175 1134 293 1164">Swertisin</p> </div> <div data-bbox="659 889 1089 1112">  <p data-bbox="651 1134 833 1164">Corniculatin A</p> </div> </div>		<p data-bbox="1141 421 1446 451">Sarma and Kumari (2014)</p>
<p data-bbox="164 1195 1117 1470">Tannins, saponins, terpenoids, glycosides, phenolic compounds, ferritin in integumentary cells of ovule, immature plastids, oxalic acid, ?- sitosterol, betulin, 4-hydroxybenzoic acid, ethyl gallate, 7,5'-dimethoxy- 3, 5, 2'-trihydroxyflavone, apigenin, glucopranoside, Flavanoids, tannins, phytosterols, phenol, glycoseides, fatty acids, galacto-glycerolipid and volatile oil, Phenoic compounds, gallic acid, flavonols and flavonoids, C-glycosylfavones , harmine (7- methoxy-1- methyl-beta-carboline) and harmaline (3, 4dihydroharmine),heptadecyl-5-methoxy-phenol, Alkaloids, carbohydrates and glycosides, phytosterols, phenolic compounds, aminoacids, flavonoids, volatile oil, proteins.</p> <div style="display: flex; justify-content: space-around; margin-top: 20px;"> <div data-bbox="183 1517 626 1847">  <p data-bbox="175 1859 318 1889">β-sitosterol</p> </div> <div data-bbox="659 1517 1089 1847">  <p data-bbox="651 1859 740 1889">Betulin</p> </div> </div>		<p data-bbox="1141 1195 1446 1364">Raghavendra <i>et al.</i>, (2006), Qureshi <i>et al.</i>, (2009), Manna <i>et al.</i>, (2010), Kumar <i>et al.</i>, (2012), Mohan <i>et al.</i>, (2015), Panda <i>et al.</i>, (2016)</p>

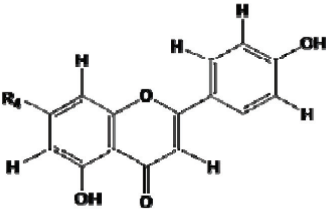
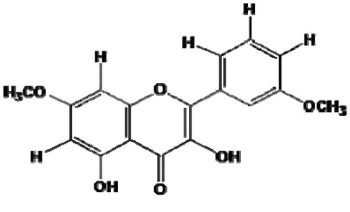
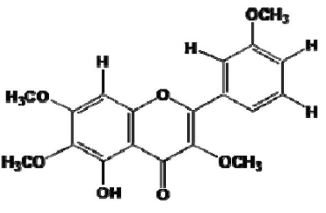
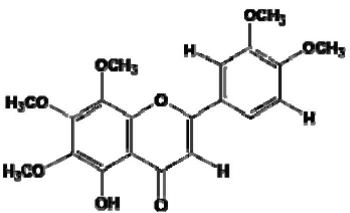
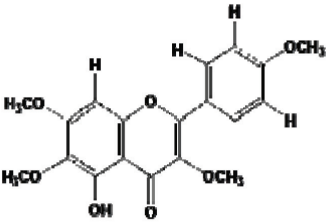
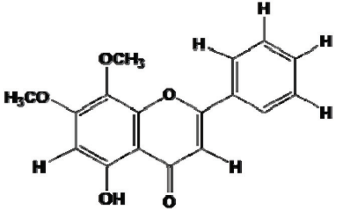
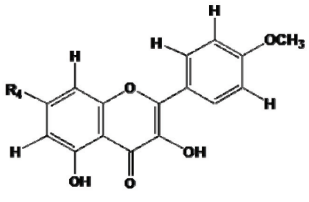
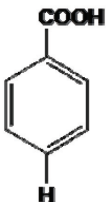
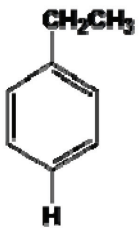
Chemical structure of reported phytoconstituents	References
 <p>Apigenin</p>	
 <p>7,5'-dimethoxy-3,5,2'-trihydroxyflavone</p>	
 <p>4', 5-hydroxy-3,6,7-trimethoxylavone</p>	
 <p>5-hydroxy-3',4',6,7,8-pentamethoxyflavone</p>	
 <p>5-hydroxy-3,6,7,4'-tetra-methoxylavone</p>	
 <p>5-hydroxy-7,8-dimethoxylavone</p>	
 <p>3,3',5,7-trihydroxy-4'-methoxylavone</p>  <p>4-hydroxybenzoic acid</p>  <p>ethyl gallate</p>	
<p><i>Oxalis debilis</i></p>	
<p>Ascorbic acid, crude fibre, phenolic compounds, glycosides, carbohydrate, tannins, terpenoids, alkaloids and saponin in aqueous extract</p>	<p>Sarma <i>et al.</i>, (2015), Panda <i>et al.</i>, (2016), Junejo <i>et al.</i>, (2016)</p>
<p><i>Oxalis triangularis</i></p>	
<p>Ten fatty acid alkyl esters, methyl/ethyl linoleate and linolenate</p>	<p>Uh <i>et al.</i>,(2010)</p>

Table 2

Effect of crude extract of *O. corniculata* and *O. debilis* on different microorganisms

Solvent systems used	Pathogen used for antimicrobial test	Antimicrobial effect	References
<i>Oxalis corniculata</i>			
Methanol and ethanol extracts showed significant activity	<i>Xanthomonas</i> and fourteen human pathogenic bacteria.	Positive effect	Raghavendra <i>et al.</i> , (2006)
Aqueous extract	<i>S. aureus</i> and <i>E. coli</i>	Positive effect	Handali <i>et al.</i> , (2011)
Methanolic extract	<i>Bacillus subtilis</i> , <i>Streptococcus epidermis</i> , <i>Pseudomonas aeruginosa</i>	Most effective against <i>Bacillus subtilis</i> and <i>Streptococcus epidermis</i> as compare to <i>Pseudomonas aeruginosa</i>	Panda <i>et al.</i> , (2016)
Activity of aqueous ethanol and ethyl ether	<i>Staphylococcus faecalis</i> , <i>Escherichia coli</i> , <i>P. vesicularis</i> , <i>Aeromonas hydrophilia</i> , <i>Stphylococcus cohni</i> , <i>Serratia ficaria</i> , <i>S. typhi</i>	Effective against all tested organism	Mohan <i>et al.</i> , (2015)
<i>Oxalis debilis</i>			
Methanol extract	<i>Bacillus subtilis</i> and <i>Pseudomonas aeruginosa</i>	Highly effective against <i>Bacillus subtilis</i> and <i>Pseudomonas aeruginosa</i>	Panda <i>et al.</i> , 2016

4. Antibacterial activity

The antimicrobial activities of crude extracts of *Oxalis* species, as reported earlier, have been presented in Table 2. Methanolic extract of *O. debilis* was found to be highly effective against *Bacillus subtilis* and *Pseudomonas aeruginosa* (Panda *et al.*, 2016). There are not much work of antibacterial activity of *O. debilis*. And *O. triangularis* except our work described above (Table 2, Fig. 5).

5. Pharmacological activities

The methanolic, petroleum ether and ethyl acetate extract of *O. corniculata* leads to death of *Eisenia foetida* worm and paralysis as reported by Dighe (2014). It is reported by Chitwood (2002) and Leando (2004) that ethanolic extract of *O. corniculata* has nematotoxic activity against phytoparasitic nematodes. Similarly, in case of *O. debilis*, single dose administration of hydro-alcoholic extract up to the level of 5000 mg/kg body weight has showed no

toxic symptoms or mortality over any animals up to 14 days of the experimental observations. With the treatment of high dose of hydro-alcoholic extract of *O. debilis*, there is no reduction in body weight of the test animals during the period of experiment, which needs further investigation to identify the responsible component of the plant extract in enhancement of weight (Junejo *et al.*, 2016).

6. Anti-oxidant activity

The most common antioxidants present in herbs and fruits are vitamins C and E, carotenoids, flavonoids and thiol compounds (-SH) etc. Natural antioxidants supplementation through a balanced diet containing adequate herbs could be much more effective than the individual antioxidant uptake such as vitamin C or vitamin E. Methanolic extract of whole plant of *O. corniculata* has been reported to be having significant antioxidant DPPH and nitric oxide radical scavenging activity. The extract has

inhibitory effect against lipid peroxidation. The presence of high phenol content, flavonoids and flavonols has been reported, which is largely responsible for the plant's anti-oxidant activity. Extract also showed *in-vitro* anti-inflammatory activity by inhibiting the heat induced albumin denaturation and Red Blood Cells (RBC) membrane stabilization with the IC₅₀ values of 288.04 and 467.14 µg/ml respectively (Sakat *et al.*, 2010). Similarly, *O. debilis* also possesses anti-oxidant activity. The total phenol content of *O. debilis* was found to be ~1.6 fold higher as compared to *O. corniculata* (Sarma *et al.*, 2015). The ascorbic acid content also reported higher in *O. debilis* (110.75 mg/100 gm) in comparison to *O. corniculata* (92.20 mg/100 gm). *O. debilis* had higher antioxidant activity (IC₅₀=25.82 µg/ml) than *O. corniculata* (IC₅₀=73.67 µg/ml) (Sarma *et al.*, 2015). However, there is no published report yet on anti-oxidant properties of *O. triangularis*.

7. Cytogenetics and phylogeny of *Oxalis* species

A number of publications are available on the floral morphology, pollen viability and DNA ploidy level of *Oxalis* species (Castro *et al.*, 2007; Luo *et al.*, 2006; Tsai *et al.*, 2010). *Oxalis pes-caprae* is a widespread invasive weed, which is reported as heterostylous with trimorphic flowers and a self- and morph-incompatible reproductive system in its native habitat (southern Africa) but in most of the areas invaded, only a pentaploid short-styled morphotype that reproduces mainly asexually by bulbils is reported. The low or null sexual reproduction success of this species in the area of invasion studied seems related with the high frequency of monomorphic populations, the unequal proportion of floral morphs in dimorphic populations and the presence of different ploidy levels between short-styles and long-styled morphs (Castro *et al.*, 2007). As generally perceived, the successful establishment of exotic species through vegetative propagation has been largely correlated with their invasion success (Godfrey *et al.*, 2004; Lloret *et al.*, 2005). When a single floral morphs is introduced in new area, the sexual attributes to the fitness of the newly established plant or population is low or null as reported in *Oxalis pes-caprae* (Castro *et al.*, 2007), *O. debilis* (Luo *et al.*, 2006) and *O. corymbosa* (Tsai *et al.*, 2010).

Cytogenetic studies revealed a great deal of variability in the basic number of chromosomes (from $x = 5$ to $x = 12$), as well as variation in the degree of ploidy level, ranging from $2x$ to $8x$ (Marks, 1956; Cronquist, 1981; de Azkue and Martínez, 1983, 1988 & 1990). Phylogenetic analysis of ncpGS sequences give a comparison with those of the internal transcribed spacer of nuclear ribosomal DNA (ITS) from different species of the genus *Oxalis* (Mshwiller *et al.*, 1999). *O. corniculata* is octoploids with $2n = 8x = 48$. Pollen

germination, pollen tube development, fruit and seed production, seed germination and offspring ploidy levels have been analysed after controlled hand-pollinations to assess self- and morph-incompatibility and production of viable gametes by the $5x$ S-morph (Costa *et al.*, 2013). According to Tosto and Hopp (2008), the RFLP characterization of nuclear r-DNA units of *O. tuberosa* and four selected diploid species through EcoRI, BamHI, EcoRI and BamHI (double digestion) and EcoRV has produced restriction map for EcoRI and BamHI. Phylogenetic results suggest that the American bulb-bearing *Oxalis* originated in southern South America, dispersed repeatedly to North America, and had multiple transitions from tristylous to distylous (Gardner *et al.*, 2012). Amplifications of the ITS/5.8S region from *Oxalis* species normally resulted in a single product of 700 bp, typical in size for angiosperms (Baldwin *et al.*, 1995). Meiotic studies recorded normal bivalents in *O. bupleurifolia* ($n=5$), *O. dentata* ($n=7$), *O. namaquana* ($n=14$) and *O. purpurea* ($n=20+2$ univalents). Though cytological studies and determination of ploidy levels in *O. cuneata* ($2n=12$), *O. cathera* ($2n=14$), *O. massoniana* ($2n=14$), *O. hirta* ($2n=30$), *O. imbricata* ($2n=40$) and *O. purpurea* ($2n=42$) have been reported by Rutland (1941), there is no report on cytology and ploidy study of *O. debilis* and *O. triangularis*.

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