

Chemical Composition and Biological Anti Microbial Activity of Some Extracts from *Anemone Narcissiflora* Subsp. *Narcissiflora*



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ABSTRACT

The antioxidant and antimicrobial activities of essential oils, methanolic and chloroform extracts from different parts of *Anemone narcissiflora* subsp. *narcissiflora* from Kumarganj Ayodhya were studied. Antioxidant activities were measured employing free radical, 2, 2-diphenyl- 1-picrylhydrazyl¹ (DPPH), scavenging ability of the samples. Total phenolic substance was measured for only methanol extracts. The extracts showed moderate free radical scavenging activity. Methanolic leaf extracts showed stronger inhibitory activity against DPPH radical with an SC₅₀ values (the antioxidant concentration to achieve 50% radical scavenging) of 29 ug/mL. the antimicrobial activity was studied by the agar dilution minimal inhibitory concentration (MIC) assay and agar well diffusion assay using seven bacteria, *Escherichia coli*, *Klebsiella pneumonia*, *Yersinia pseudotuberculosis*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *staphylococcus aureus* and *Bacillus cereus*, and two yeast-like fungi, *candida tropicalis* and *candida tropicalis*. Methanolic and chloroform extracts were effective against the bacteria except for *B. Cereus* and the fungi. The MIC values for the extracts ranged from 12.5 to 100 ug/mL, in many cases indicating better antibacterial activity as compared to the standard antibacterial ampicillin. In contract, the essential oils from the plant showed slight antimicrobial activity only on *B. cereus* at 500 ug/mL.

Keywords : *Anemone narcissiflora*, antioxidant, antimicrobial, DPPH, essential oils

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Introduction - *Anemone* L. (Ranunculaceae) is represented with eight native species in Turkiye [1]. They are all rhizomatous and their rhizomes are exported by the local plant collectors [2]. Flowers together with the upper part of *A. coronaria* L. are used in Anatolian folk medicine against wound [3]. *A. nemorosa* L., is a poisonous plant, is used in Bulgaria. Its poisonous effect reduces after drying [4].

A. narcissiflora is a rhizomatous perennial herb, distributed in North-East Anatolia has two subspecies, *A. narcissiflora* subsp. *narcissiflora* and *A. narcissiflora* subsp. *willdenowii* (Boiss.) Davis [5]. Several Anemone species are naturally distributed in Rize, Turkiye, where the most famous and the most expensive honey type. Anemone species are found in Anzer plateau and are expected to be an important floral source for Anzer honey productions. Anzer honey has curative effects against many illnesses such as farangitis,, tonsillitis, ulceration, heart and vascular diseases, infertility, cancer, anemia and skin care [6].

Antioxidants, which can inhibit or delay the oxidation of an oxidizable substrate in a chain reaction, therefore, appear to be very important in the protection of many diseases [7,8]. Several antioxidants compounds synthesized by plants as secondary products, especially phenolics, could play a major role in enhancing the antioxidant system, since they behave as reactive oxygen species scavengers, metal chelators and enzyme modulators [9]. Several antioxidant methods have been developed to evaluate the antioxidant capacity of the biological samples, the most widely used antioxidant methods involve the generation free radicals and their concentration is monitored as the present antioxidants scavenge them. Radical formation and the following scavenging are applied in DPPH measurements [7].

The aim of this study was to determine biological activities of the essential oils, methanolic and chloroform extracts of the flowers, stems and leaves of the plant to elaborate and evaluate their potential medicinal use.

Materials and Methods

Plant Material- *A narcissiflora* subsp. *narcissiflora* plants at flowering stage were collected from Kumarganj Ayodhya Anzer plateau, alpine meadows, 2650 m, Rize-Ikizdere Turkiye, in June 2004. The taxonomic identification of plant materials was confirmed by a senior professor plant taxonomist Dr. N.B. Singh in the Department of Botany, Allahabad University Allahabad (U.P.) . The Plant were dried in shadow, and the leaves, flowers, and stems were separated and ground. The voucher specimen has been deposited at the Herbarium of the Department of Biology, Karadeniz Technical University, Trabzon, Turkiye.

Isolation of the Essential Oil- The essential oil of air-dried powders was obtained through hydro-distillation (15g of powdered plant material in 1 L pure water, 3 j) by using a Clevenger-type apparatus with ice bath for cooling system. The oils were taken by dissolving in high performance liquid chromatography (HPLC)-grade n-hexane (0.5mL) and kept at 4 °C at CDRI Lucknow in a sealed brown vial until tested for biological activities.

Preparation of the Methanol and chloroform Extracts

Dried and powdered samples (25 g) were extracted successively with 250 mL of methanol and chloroform by using a Soxhlet extractor for 6 h at a temperature not exceeding the normal boiling point of the solvent. The extracts were filtered

using Whatman No 1 filter paper and then evaporated to dryness at 40 °C using a rotary evaporator. The methanolic extracts were dissolved in dimethyl sulfoxide (DMSO) and the chloroform extracts were dissolved in chloroform: DMSO solvent mixture (9:1) for antimicrobial and antioxidant tests.

Antimicrobial Activity- All the test microorganisms, seven bacteria and two yeast-like fungi, were obtained from Refik Saydam Hifzissihha Institute (Ankara, Turkiye) and were as follows: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* 'ATCC 13883, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas aeruginosa* ATCC 10145, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* 702 ROMA, *Candida albicans* ATCC 60193, *Candida tropicalis* (Yeast-like fungus) ATCC 13803. The essential oils and the extracts from the flower, leaf and stem samples were diluted with the solvents to prepare sample stock solutions of 1000 ug/mL.

Results and Discussion

Antimicrobial activity The *in vitro* antimicrobial activity of *A. narcissiflora* subsp. *narcissiflora* methanol and chloroform extracts were tested by using the agar dilution MIC assay with the microorganisms as seen in Methanolic and chloroform extracts were found to have a good activity against all microorganisms except for *B. Cereus*. Particularly, both methanolic and chloroform extracts of leaves and flowers exhibited a stronger antimicrobial activity. In general, weaker (MIC : 100 ug/mL) antimicrobial activity was observed for nonpolar solvent in fungi, but methanolic extract showed higher (MIC : 25 ug/mL.) antifungal activity. On the other hand, both methanolic and chloroform extracts of all the three parts of the plant exhibited a good activity against *E. faecalis*. *Enterococcus* species cause complicated urinary tract infections, bacteremia, endocarditis, intra-abdominal and pelvic infections, wound and soft tissue infections, neonatal sepsis, and rarely meningitis. *E. faecalis* is the most common cause of enterococcal infections (85-90%) [9, 10]. In addition, methanolic and chloroform extracts inhibited *Y. pseudotuberculosis* bacterial growth, and chloroform extract was the most effective. *Y. pseudotuberculosis* and *P. aeruginosa* cause infections in humans. It is primarily a zoonotic infection with variable hosts, including domestic and sylvatic animals. The condition has been associated with food-borne infections [11].

The antimicrobial activities of the essential oils of *A. narcissiflora* subsp. *narcissiflora* were tested *in vitro* by using the agar-well diffusion method (table-2) the essential oils showed slight antimicrobial activity against *B. cereus*. However, no antimicrobial activity was observed against the other six bacteria and two yeast like-fungi. Interestingly while methanolic and chloroform extracts were not effective against *B. cereus* essential oils were effective only on this bacterium. *B. cereus* causes a toxin-mediated food poisoning. *B. cereus* can also cause local skin and wound infections, ocular infections, fulminant liver failure, and invasive diseases, including bacteremia, endocarditis, osteomyelitis, pneumonia, brain abscess, and meningitis [10].

Table No. 2

Screening for antimicrobial activity of the essential oils
from *A. Narcissiflora* subsp. *narcissiflora* (500 ug/mL).

Samples	Microorganisms and inhibition zone (mm)								
	Ec	Kp	Yp	Pa	Ef	Sa	Bc	Ca	Ct
Flowers	-	-	-	-	-	-	+	-	-
Leaves	-	-	-	-	-	-	+	-	-
Stems	-	-	-	-	-	-	+	-	-
Hexane	-	-	-	-	-	-	-	-	-
Caftazidime (10ug)	+++	+++	+++	+++	+++	+++	+++	-	-
Triflucan (5 ug)	-	-	-	-	-	-	-	+++	+++

The results can be considered as the first detailed document on the in vitro and antimicrobial activity and chemical composition of *A. narcissiflora* subsp. *narcissiflora*. Particularly, the methanolic extracts of *A. narcissiflora* subsp. *narcissiflora* can be potentially useful source of natural antimicrobial agents and antioxidant principles to be used as nutraceuticals as well as in herbal medicine.

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