wjpmr, 2024, 10(1), 256-261

SJIF Impact Factor: 5.922

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

STUDIES ON GC-MS ANALYSIS FOR BIOACTIVE COMPOUNDS IN ETHYL ACETATE EXTRACTS OF *ALOE VERA* L. (*IN VIVO* AND *IN VITRO* REGENERATED) INNER GEL

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Article Received on 21/11/2023 **Article Revised on 11/12/2023** **Article Accepted on 01/01/2024**

ABSTRACT

Plant-derived medicines are the most widely used medicines in the world today. The use of herbs and plants as first medication is practiced universally. Every culture on earth, through written or oral tradition, has relied on the vast variety of natural chemistry found in healing plants for their therapeutic properties. Nearly 5.1 billion people worldwide employ natural plant-based remedies as their primary medicines for both acute and chronic health problems. Plants with therapeutic potential may be defined as any plant that can be put to culinary and/or medicinal use. *Aloe vera* L. has been used for medicinal purposes in several cultures for millennia: Greece, Egypt, India, Mexico, Japan, and China. The products prepared from *Aloe* leaves have multiple properties such as emollient, purgative, antibacterial, antioxidant, antifungal and antiseptic. Present investigation was done to study the quantitative and spectral analysis (GC-MS) for medicinally important phytochemicals (especially antimicrobials including antibacterial as well as antifungal agents) present in inner gel ethyl acetate extracts from *in vivo* and *in vitro* regenerated *Aloe vera* L. The GC-MS analysis of ethyl acetate extracts of inner gel from *in vivo* and *in vitro* regenerated *Aloe vera* L. showed the presence of various secondary metabolites such as phenolics, alkaloids, flavonoids, tannins, saponins and steroids. Such secondary metabolites from plant sources have been reported to be potent antimicrobial agents.

KEYWORDS: *Aloe vera* L., quantitative & spectral analysis, GC-MS analysis, secondary metabolites, phytochemicals, ethyl acetate extracts and natural antimicrobials.

INTRODUCTION

Plant-based antimicrobials represent a vast untapped source for medicines and further exploration of plant antimicrobials needs to occur. Whether, historically medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs.[1] Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated.[2]

There has been an increase in demand for the phytopharmaceuticals all over the world. *Aloe vera* L. leaf gel works as a strong antioxidant as well as an important antimicrobial agent. As we can see in current era, most of the microbial strains are continuously being resistant to most of the synthetic/allopathic drugs, therefore, there is a necessity of the search for the drug of natural or herbal origin. Herbal medications in particular have seen a revival of interest due to a perception that there is a lower incidence of adverse reactions to plant preparations compared to synthetic pharmaceuticals. Coupled with the reduced costs of plant preparations, this makes the search for natural therapeutics an attractive option. *Aloe vera* L. might be a wonder herb as antioxidant and antimicrobial.[3]

The leaf pulp and liquid fraction of *Aloe vera* L. act against various microorganisms. The Chinese describe aloe‘s skin and the inner lining of its leaves as a cold, bitter remedy which is downward draining and used to clear constipation due to accumulation of heat; the gel is considered cool and moist. In Ayurvedic medicine of India, *Aloe* is used internally as a laxative, antihelminthic, hemorrhoid remedy and uterine stimulant (menstrual regulator); in combination with licorice root, to treat eczema or psoriasis. Furthermore, activity against a variety of infectious agents has been attributed to *Aloe*

vera L. for instance, antibacterial, antiviral and antifungal.[3]

GC-MS method used for the analysis of the obtained extracts can be an interesting tool for testing the amount of some active principles in herbs used in cosmetic, drugs, pharmaceutical or food industry, environmental and forensic applications. It combines two analytical techniques to a single method of analysing mixtures of chemical compounds. Gas chromatography separates the components of the mixture and mass spectroscopy analyses each of the components separately.[4]

Therefore, the current study was aimed to assess the phytochemical constituents in ethyl acetate extracts of *in vivo* and *in vitro* regenerated *Aloe vera* L. inner gel.

MATERIALS AND METHODS

Preparation of crude extract

Leaves of the *Aloe vera* L. were collected from the already *in vitro* propagated and properly acclimatized 9-12 months old plants. *In vitro* propagation was the previous phase of our study to produce quality plant material to meet industrial requirement. Simultaneously leaves from 9-12 months old *in vivo* grown *Aloe vera* L. plants were also collected. Freshly collected *Aloe vera* L. leaves were washed with distilled water, followed by disinfecting with ethanol 70%. Later, upper green skin/rind of leaves was removed and latex was cut into small pieces and all the leaf material collected was homogenized. The homogenized material was extracted with ethanol (95%). The ethanol from the extracted leaf materials was evaporated at 65^{o}C temperature in water bath. The solvent was completely removed and dried to get powder and finally all the powdered plant material of inner gel form *in vivo* as well as *in vitro* regenerated *Aloe vera* L. were used for the preparation of solvent extracts.

Preparation of solvent extracts

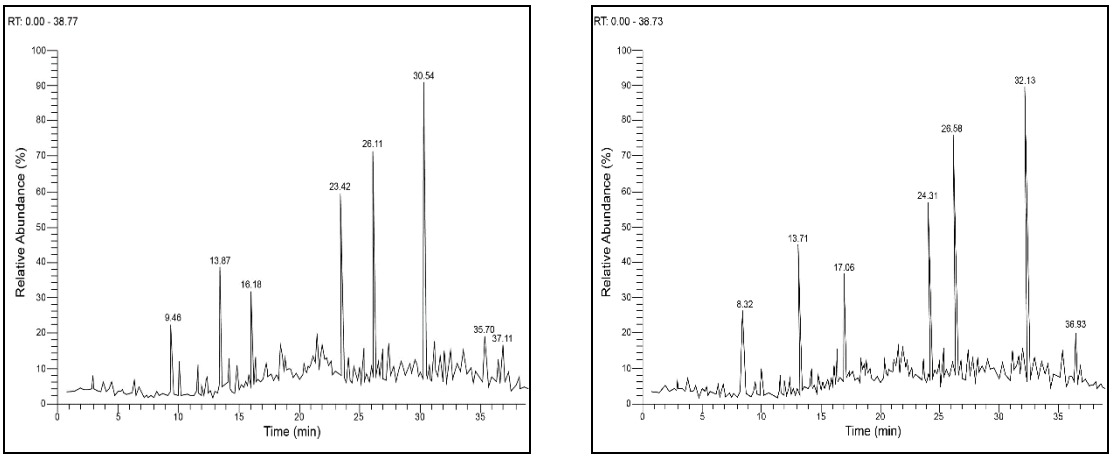
Extracts were prepared using the modified method of Case.[5] 1:3 (w/v) ratios were used for the powdered inner gel leaf material and ethyl acetate as solvent for extract preparation. The pulverized leaves material was mixed with sufficient quantity of solvent i.e. ethyl acetate and then it was kept in rotary shaker at 100 rpm overnight and filtered with Whatman No.1 filter paper and subsequently subjected to lyophilization at – 47.5ºC. The dried extracts thus obtained was weighed, transferred into separate vials and preserved at 4ºC for future analysis.

GC-MS analysis

The ethyl acetate extract of *in vitro* and *in vivo* grown *Aloe vera* L. inner gel was analysed by a gas chromatography apparatus using a DB-S capillary column (30m) equipped with MS detector with helium as a carrier gas at a flow rate of 1ml/minute. All the compounds were identified by comparison with the standards, and also matched with the in-built libraries.

RESULTS

GC-MS analysis was done to study the quantitative phytochemical properties in ethyl acetate extracts of *in vivo* and *in vitro* regenerated *Aloe vera* L. inner gel extracts as these were showing the maximum antimicrobial activities. The GC-MS output of the ethyl acetate extracts of *in vivo* grown *Aloe vera* L. inner gel showed six major peaks at retention times 9.46, 13.87, 16.18, 23.42, 26.11 and 30.54 minutes (Fig. 1a). The GC-MS outputs of the ethyl acetate extracts of *in vitro* regenerated *Aloe vera* L. inner gel was also showing six major peaks at retention times 8.32, 13.71, 17.06, 24.31, 26.58 and 32.13 minutes (Fig. 1b).



***(a) in vivo* grown** **(b) *in vitro* regenerated**

Fig. 1: GC-MS spectrum of ethyl acetate extracts of *in vivo* and *in vitro* regenerated *Aloe vera* L. inner gel.

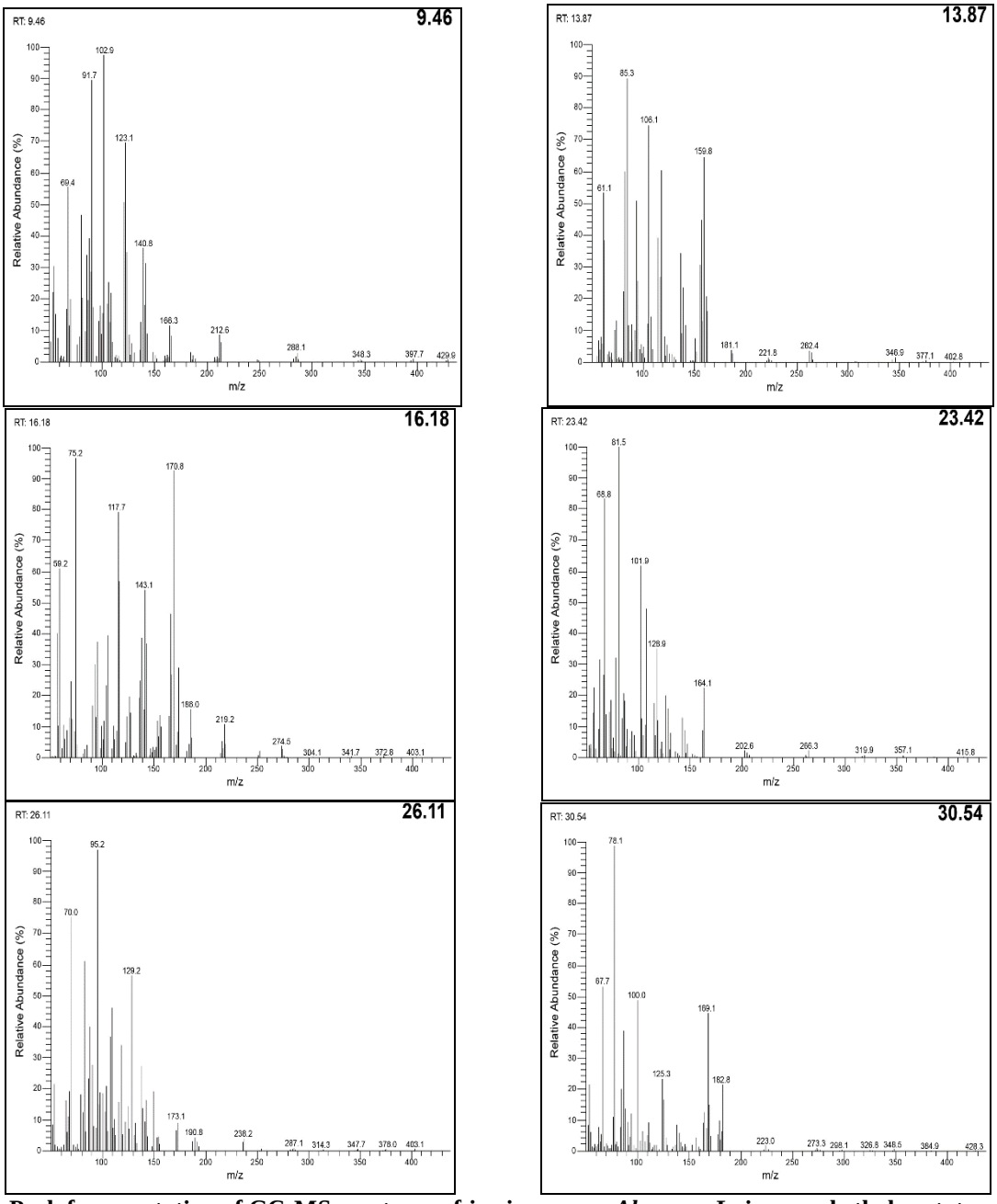
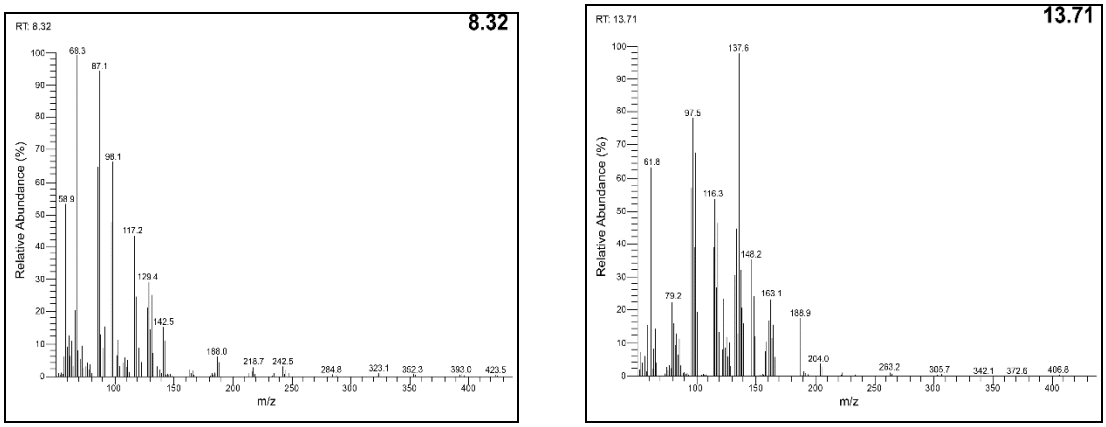


Fig. 2: Peak fragmentation of GC-MS spectrum of *in vivo* grown *Aloe vera* L. inner gel ethyl acetate extract.



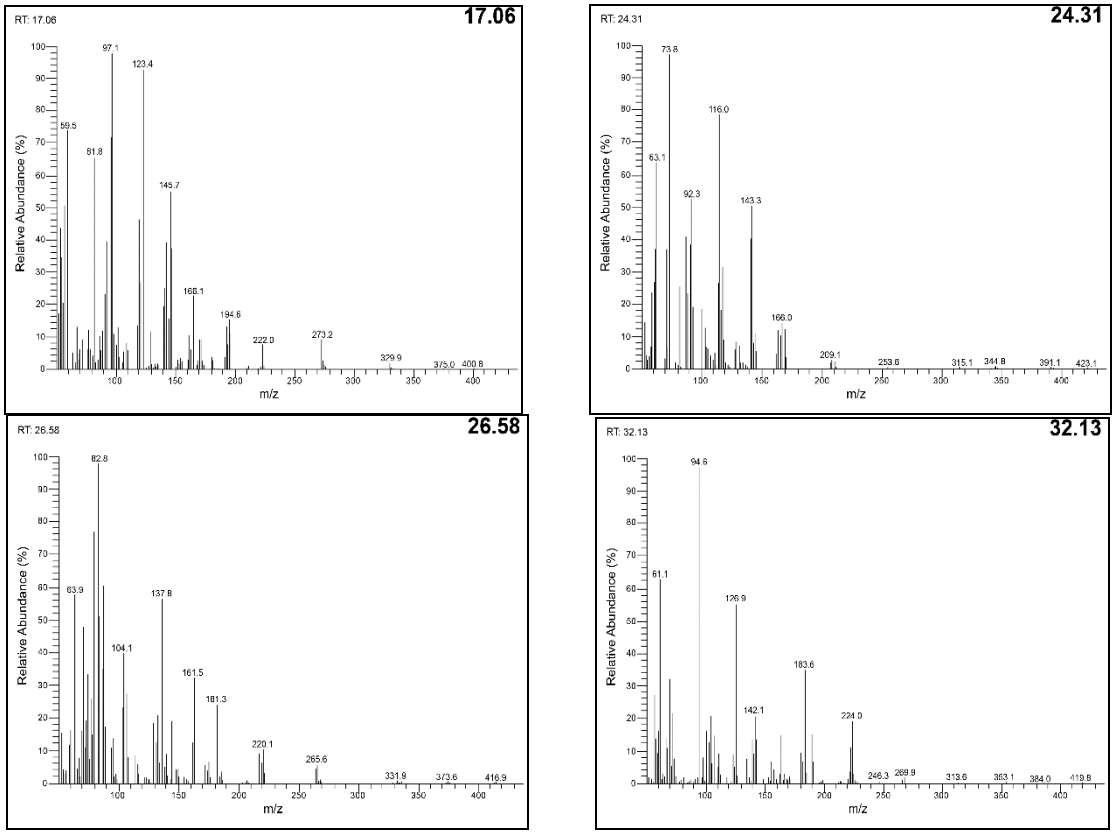


Fig. 3: Peak fragmentation of GC-MS spectrum of *in vitro* regenerated *Aloe vera* L. inner gel ethyl acetate extract.

The mass spectrum of ethyl acetate extract of *in vivo* grown *Aloe vera* L. inner gel at retention time 9.46 showed five characteristic (M-15), (M-29), (M-14), (M-14) and (M-28) peaks at m/z 212.6, 140.8, 123.1, 102.9 and 91.7 respectively. This indicated the presence of– CH_3, – N, – CH_2, – CH_2 and – CO group containing compound in this fraction (Fig. 2).

The mass spectrum of ethyl acetate extract of *in vivo* grown *Aloe vera* L. inner gel at retention time 13.87 showed four characteristic (M-29), (M-17), (M-15) and (M-14) peaks at m/z 262.4, 159.8, 106.1 and 85.3 respectively. This indicated the presence of –N, –OH, – CH_3 and – CH_2 group containing compound in this fraction (Fig. 2).

The mass spectrum of ethyl acetate extract of *in vivo* grown *Aloe vera* L. inner gel at retention time 16.18 showed six characteristic (M-14), (M-15), (M-15), (M-45), (M-14) and (M-14) peaks at m/z 219.2, 186.0, 170.8, 143.1, 117.7 and 75.2 respectively. This indicated the presence of–CH_2, –CH_3, –CH_3, –COOH, –CH_2 and – CH_2 group containing compound in this fraction (Fig. 2).

The mass spectrum of ethyl acetate extract of *in vivo* grown *Aloe vera* L. inner gel at retention time 23.42 showed five characteristic (M-15), (M-28), (M-14), (M-17) and (M-28) peaks at m/z 164.1, 128.9, 101.9, 81.5 and 68.8 respectively. This indicated the presence of – CH_3, – CO, – CH_2, – OH, and – CO group containing compound in this fraction (Fig. 2).

The mass spectrum of ethyl acetate extract of *in vivo* grown *Aloe vera* L. inner gel at retention time 26.11 showed four characteristic (M-14), (M-14), (M-28) and (M-17) peaks at m/z 190.8, 173.1, 129.2 and 95.2 respectively. This indicated the presence of –CH_2, –CH_2, – CO, and – OH group containing compound in this fraction (Fig. 2).

The mass spectrum of ethyl acetate extract of *in vivo* grown *Aloe vera* L. inner gel at retention time 30.54 showed five characteristic (M-15), (M-29), (M-27), (M-15) and (M-14) peaks at m/z 182.8, 169.1, 125.3, 100.0 and 78.1 respectively. This indicated the presence of– CH_3, – N, – HCN, – CH_3, and – CH_2 group containing compound in this fraction (Fig. 2).

The mass spectrum of ethyl acetate extract of *in vitro* regenerated *Aloe vera* L. inner gel at retention time 8.32 showed five characteristic (M-15), (M-14), (M-27), (M-28) and (M-14) peaks at m/z 242.5, 188.0, 129.4, 87.1 and 68.3 respectively. This indicated the presence of – CH_3, – CH_2, – HCN, – CO, and – CH_2 group containing compound in this fraction (Fig. 3).

The mass spectrum of ethyl acetate extract of *in vitro* regenerated *Aloe vera* L. inner gel at retention time 13.71 showed four characteristic (M-14), (M-29), (M-15) and

(M-45) peaks at m/z 204.0, 163.1, 137.6 and 97.5 respectively. This indicated the presence of $-CH_2$, $-N$, $-CH_3$, and $-COOH$ group containing compound in this fraction (Fig. 3).

The mass spectrum of ethyl acetate extract of *in vitro* regenerated *Aloe vera* L. inner gel at retention time 17.06 showed eight characteristic (M-15), (M-15), (M-28), (M-17), (M-29), (M-14), (M-14) and (M-27) peaks at m/z 273.2, 222.0, 194.6, 166.1, 145.7, 123.4, 97.1 and 59.5 respectively. This indicated the presence of $-CH_3$, $-CH_3$, $-CO$, $-OH$, $-N$, $-CH_2$, $-CH_2$ and $-HCN$ group containing compound in this fraction (Fig. 3).

The mass spectrum of ethyl acetate extract of *in vitro* regenerated *Aloe vera* L. inner gel at retention time 24.31 showed five characteristic (M-28), (M-29), (M-17), (M-14) and (M-15) peaks at m/z 166.0, 143.3, 116.0, 92.3 and 73.8 respectively. This indicated the presence of CO, $-N$, $-OH$, $-CH_2$, and $-CH_3$ group containing compound in this fraction (Fig. 3).

The mass spectrum of ethyl acetate extract of *in vitro* regenerated *Aloe vera* L. inner gel at retention time 26.58 showed six characteristic (M-14), (M-17), (M-45), (M-28), (M-15) and (M-15) peaks at m/z 265.6, 220.1, 161.5, 137.8, 104.1 and 82.8 respectively. This indicated the presence of $-CH_2$, $-OH$, $-COOH$, $-CO$ $-CH_3$, and $-CH_3$ group containing compound in this fraction (Fig. 3).

The mass spectrum of ethyl acetate extract of *in vitro* regenerated *Aloe vera* L. inner gel at retention time 32.13 showed four characteristic (M-15), (M-14), (M-28) and (M-29) peaks at m/z 224.0, 183.6, 126.9 and 94.6 respectively. This indicated the presence of $-CH_3$, $-CH_2-CO$ and $-N$ group containing compound in this fraction (Fig. 3).

DISCUSSION

GC-MS analysis mainly used to identify and analyse the components present in the extracts and also provides insight into further development of research [6]. In present study the GC-MS analysis of the ethyl acetate extracts of *in vivo* and *in vitro* regenerated *Aloe vera* L. inner gel extracts were carried out to identify the nature of active components present. The outcomes of quantitative phytochemical studies and spectral analysis (GC-MS analysis) showed that the *Aloe vera* L. extracts contain phenolic, alkaloid, flavonoid, tannin, saponin and steroid type of compounds.

Rajamohan *et al*.,[7] worked on antioxidant and antimicrobial activities in the flowers of *Calotropis gigantea* and found the presence of phytochemicals such as alkaloids, tannins, phenol, flavonoids, sterols, anthraquinones, proteins and quinines in flower extract of. The GC-MS analysis of the flower extract revealed the presence of four major compounds.

Phytochemical and GC-MS analysis of *Hygrophila auriculata* plant extracts revealed the presence of various active components such as alkaloids, tannins, glycosides, terpenoids, steroids, flavonoids and saponins when tested for their antimicrobial activities.[8]

Therefore, several studies by different workers including results and observations of the current investigation strongly supports that the active components present in *Aloe vera* L. possesses strong antimicrobial potentials.

CONCLUSION

In present study quantitative and spectral analysis (GC-MS) was carried out to detect the nature of active components present in ethyl acetate extract of *in vivo* and *in vitro* regenerated *Aloe vera* L. inner gel extracts and it confirmed the presence of phenolics, alkaloids, flavonoids, tannins, saponins and steroids. Therefore, through these results and observations of the current investigation it can be concluded that the *Aloe vera* L. inner gel extracts possesses strong antimicrobial potentials.

ACKNOWLEDGEMENT

Authors are highly thankful to Dr. B. R. Singh, Principal Scientist, IVRI, Izzatnagar Bareilly (U.P.) India, for his kind co-operation and skilful guidance and also grateful to IFTM University, Moradabad, (U.P.), India and Hindu College, Moradabad, (U.P.), India.

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