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RESEARCH PAPER

TITLE:

CHEMICAL PROFILING AND BIOANALYTICAL COMPARISON OF *MALUS DOMESTICA* VARIETIES (Gacha Apple and Kala Kulu Apple)

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CHEMICAL PROFILING AND BIOANALYTICAL COMPARISON OF *MALUS DOMESTICA* VARIETIES (Gacha Apple and Kala Kulu Apple)

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ABSTRACT

Plants for therapeutic purposes have been used since ancient times. The current study compares the cytotoxic, anti-diabetic, anti-microbial and antioxidant properties of the Kala Kulu and Gacha apples (varieties of *Malus domestica*). In aqueous extract, the yield of Gacha and Kala Kulu was 57.492% and 43.988%, respectively. Gacha apple showed maximum values for both total phenols and flavonoid contents. The Gacha apple had a higher DPPH (67.87%) value than the Kala Kulu apple (77.10%). For alpha-amylase inhibition, the mean inhibition value of the Gacha apple was higher (86.69%) than Kala Kulu apple (69.27%). For antimicrobial activity, the zones of inhibition of the Gacha apple were 0 mm and 11 mm. In comparison, that of the Kala Kulu apples were 13 mm and 10 mm against *E. coli* and *S. aureus* respectively. The percent hemolysis by Gacha apple was 0.44% while for Kala Kulu it was 0.12%. FTIR of both varieties (Gacha and Kala Kulu apple) of *Malus domestica* showed the presence of compounds like alcohols, carboxylic acids, phenols**,** alkanes, esters, fluoride, secondary and primary amides. It is concluded that the aqueous extracts of both varieties have amazing pharmacological activities. Further research is needed to fully assess the remarkable qualities and therapeutic benefits of these medicinal plants.

KEYWORDS: *Malus domestica*, alpha-amylase, hemolytic, antioxidant, antimicrobial

1. INTRODUCTION

Numerous pharmaceutical medications have been extensively employed for the management of diseases. The overuse of these medications has adverse effects on essential organs and tissues, leading to cellular toxicity, liver, gastrointestinal and heart diseases. Remedial florae is an alternative to synthetic medicines to treat a wide range of conditions including mental health issues (stress, anxiety, and depression) and cancer. Medicinal plants have fewer adverse effects than human-made drugs (**Rahman *et al*., 2022**). Throughout history, the use of medicinal plants that contain a variety of bioactive substances with therapeutic properties is evident. Approximately 80% of the global population uses plant-based medications (**Nisar *et al*., 2018**).

Malus domestica, *a* member of *the* family Rosaceae, is quite an economical fruit that is high in nutrients. Fresh apples have a high percentage of water approximately 85% and a lower sugar level of 10-12% on average. *Malus domestica* (apple), with its extensive nutritional profile, acts as an extraordinary source of antioxidants in daily diets. Apples are responsible for providing 22% of all the readily available phenolic compounds. Procyanidins, chlorogenic acids, flavonoids,

hydroxycinnamic acids, anthocyanins and quercetins are common phenolic compounds with great antioxidant potential found in apples and these chemicals are mostly concentrated in *the* skin. Some studies show that apple seeds are poisonous to human health but they have a significant amount of nutrients and are heavy in protein and fat (**Chakraborty *et al*., 2023**). The oil of apple seed has chemical and physical qualities similar to edible oils, suggesting that it is more stable and could be employed in industries of value. Furthermore, in vitro, apple seeds have cytotoxic efficacy against distinct cancer cell lines that demonstrate their anticancer potential (**Kappa *et al*., 2023**).

In terms of preventing obesity, lowering cholesterol, reducing the incidence of breast cancer and preventing stroke, the inclusion of apples in daily meals regularly can have a substantial positive impact. *M. domestica* showed several different pharmacological activities such as antioxidant, antimicrobial, anti-proliferative, anti-diabetic, anti-obesity, anti-inflammatory, cardio-protective effect, anti-asthmatic, neurodegenerative diseases effect **(**Parkinson's and Alzheimer's disease) and anti-depressant activity (**Saeed *et al*., 2023**).

Many varieties of apple found in Pakistan were studied in previous studies such as Golden Delicious, Red Delicious and many more, but limited research is being done on two commonly consumed varieties Gacha and Kala Kulu. So, the current study was designed to evaluate the antidiabetic, antiradical, antimicrobial and cytotoxic activities of Gacha (GAE) and Kala Kulu (KKAE) fruit along with structural characterization.

2. MATERIALS AND METHODS

Sample Preparation

Both varieties of *M. domestica* were collected from local retail markets in Faisalabad, Pakistan. The samples were washed, shade-dried, crushed and stored in airtight plastic bags. Extracts were prepared by soaking 50g of powder in 500 mL of distilled water at room temperature for 3 days with frequent stirring. Following filtration, samples were placed in a water bath at 52-55 °C to form semi-solid extracts that were stored in the freezer for further analysis.

The total phenolic contents (TPCs), total flavonoid contents (TFCs), antioxidant capacities and antidiabetic potential were determined as described previously (**Jabeen *et al*., 2023**). For antibacterial activity, two bacterial strains (*Staphylococcus aureus* and *E. coli*) were used. Cytotoxicity activity was measured by the hemolytic assay, as explained earlier by **Hussain *et al*. (2021).** Structural Analysis by Fourier Transform Infrared Spectroscopy (FTIR) was done on powdered pellets (**Ali *et al*., 2022**).

Statistical analysis

All tests were done in triplicate and data were represented as mean ± S.D. The data scientist (Co-author) measured the significance level by t-test (Minitab statistical software version 17).

3. RESULT

The results are presented in Table 1. Data presented as mean percentage ± S.D. that showed a non-significant difference ($p>0.05$) in total phenolic content values. In the case of TFC and antioxidant activity, data analysis

showed a significant difference ($p<0.05$) in total flavonoid content values and DPPH scavenging potentials of both varieties. Both varieties were potent inhibitors of alpha-amylase activity, as a highly significant difference ($p<0.05$) in alpha-amylase inhibition assay values was observed. Gacha apple showed no antimicrobial activity while Kala Kulu apple showed moderate inhibition against *E. coli*. Both samples exhibited moderate growth inhibitions for *Staphylococcus aureus*. Percentage hemolysis by GAE was observed to be 0.44% and that of KKAE was observed to be 0.12% (Fig.1). FTIR spectroscopy results of both varieties (Gacha and Kala Kulu) of *Malus domestica* are shown in Figure 2, Figure 3 and Table 2, respectively.

Table-1. Bioanalytical Comparison

Treatments	**Antioxidant profile**			**Anti-diabetic profile (%)**	**Antimicrobial profile (mm)**		**Cytotoxicity (%)** **Hemolytic assay**
	TPC	**TFC**	**DPPH**	**Alpha-amylase inhibition**	***E.coli***	***S. aureus***	
GAE	32.836±1.30	107.33±5.45	67.87±0.36	86.69±0.24	No activity	11	0.44±0.02
KKAE	33.846±1.02	82.37±7.80	77.10±0.24	69.27±0.19	13	10	0.12±0.1
Control	730.87±6.53	222.44 ± 2.43	89.638±0.00	81.47±0.00	27	28	94.877±0.00

Data expressed as mean ± SD, mm inhibition zones of triplicate measurements. Gacha apple extract: GAE; Kala Kulu apple extract: KKAE; TPC: mg gallic acid equivalents /100 g dry weight; TFC: mg catechin equivalents/100 g dry weight; DPPH: 2, 2-diphenyl l-picrylhydrazyl; EC: *E. coli*; SA: *S. aureus;* Positive controls: BHT; Butylated hydroxytoluene (DPPH), Acarbose (alpha-amylase inhibitory assay), Triton X-100: hemolytic assay, Ciprofloxacin: antimicrobial assay.

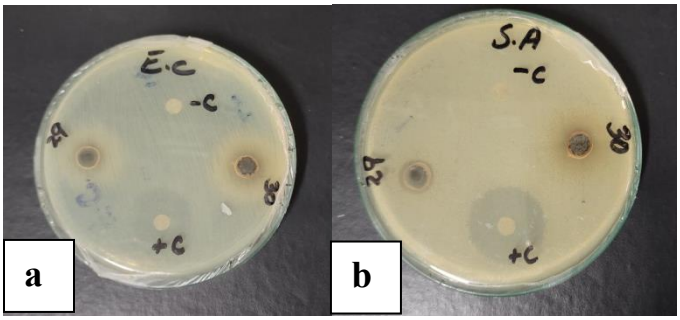

Fig. 1 Antimicrobial activity against *S. aureus* (a) and *E. coli* (b)

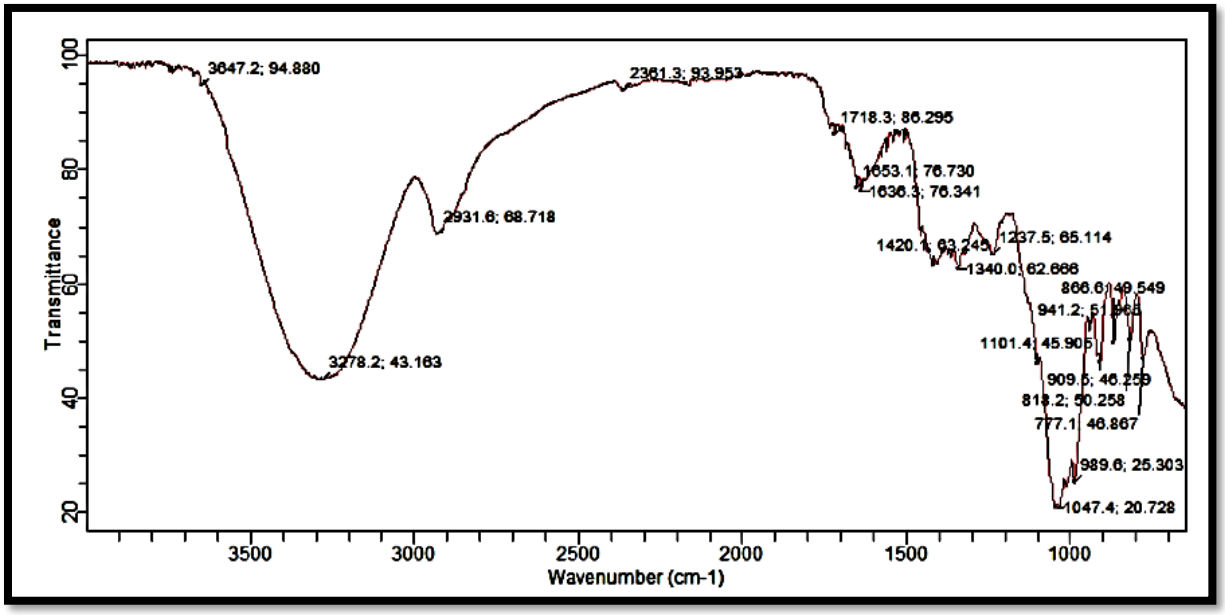

Fig. 2: FTIR spectra of *Malus domestica* (Gacha)

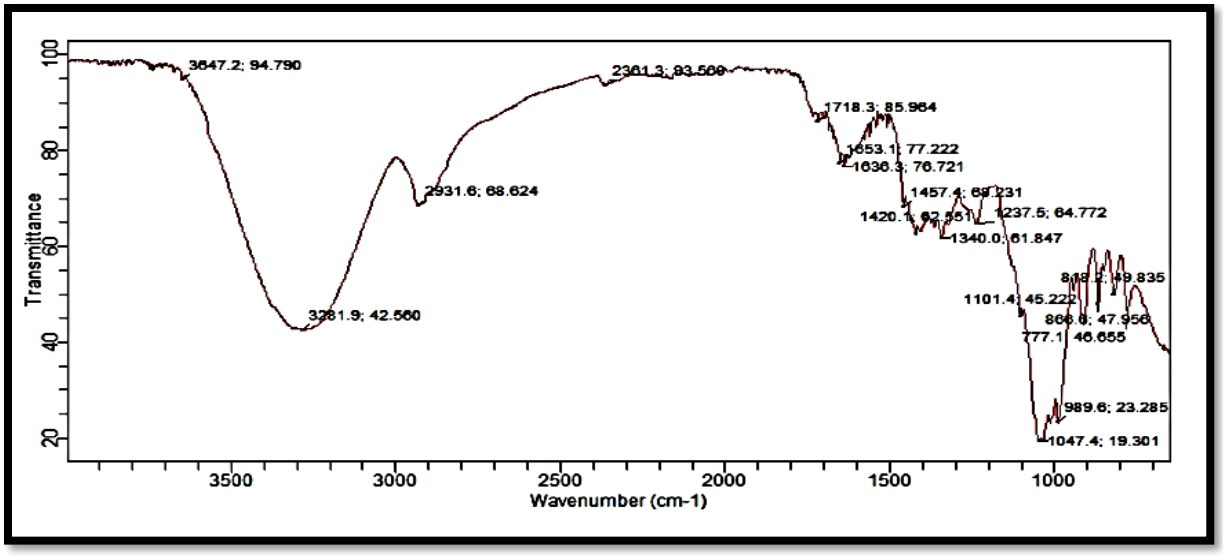

Fig. 3: FTIR spectra of *Malus domestica* (Kala Kulu)

Table-2. FTIR results

***Malus domestica* (Gacha apple)**			***Malus domestica* (Kala Kulu apple)**		
Absorption	**Compound class**	**Functional group**	**Absorption**	**Compound class**	**Functional group**
3647.2	Alcohols, phenols	O-H	3647.2	Alcohols, phenols free	O-H
3278.2	Alcohols, phenols bonded, Carboxylic acid	H-O-H	3281.9	Alcohols, phenols, H-bonded, Carboxylic acid	O-H
2931.6	Alkane, Carboxylic acid	C-H stretched O-H stretched	2931.6	Alkane, Carboxylic acid	C-H stretched O-H stretched
2361.3	Carbon dioxide	O=C=O	2361.3	Carbon dioxide	O=C=O
1718.3	Ketone Carboxylic acid	C=O	1718.3	Ketone Carboxylic acid	C=O
1653.1	Imines and oximes Amide, Alkene	C=N C=O, C=C	1653.1	Imines and oximes Amide, Alkene	C=N C=O, C=C

1636.3	Amines, amides Alkene	N-H bended C=C	1636.3	Primary and secondary amines and amides Alkene	N-H bended C=C
1420.1	Nitro	N=O	1457.4	Nitro	N=O
1340.0	Fluorides	C-X	1420.1	Nitro	N=O
1237.5	Fluorides, Amines, Alcohols,	C-X C-N, C-O	1340.0	Fluorides	C-X
1101.4	Fluorides, Amines, Alcohols, esters,	C-X C-N, C-O	1237.5	Fluorides, Amines, Alcohols, esters,	C-X C-N, C-O
1047.4	Fluorides, Amines Alcohols, esters, ethers,	C-X C-N, C-O	1101.4	Fluorides, Amines, Alcohols, esters,	C-X C-N, C-O
989.6	Alkenes	C-H	1047.4	Fluorides,Amines Alcohols	C-X, C-N C-O
941.2	Alkenes	C-H	989.6	Alkenes	C-H
909.5	Alkenes	C-H	866.6	Aromatics and alkenes	C-H
866.6	Aromatics, alkenes	C-H	818.2	Aromatics and alkenes	C-H
818.2	Alkenes	C-H	777.1	Chloride, Alkenes	C-X, C-H

4. DISCUSSION

In the current study, aqueous extract of both Gacha and Kala Kulu showed lower TPC values as compared to the previous study. **Tareen *et al*. (2021)** studied five varieties of apples i.e. Mashadi, Red Delicious, Katja, Amri and Golden Delicious. Their findings revealed that 80% aqueous methanol extract of Red Delicious (peel) showed the highest TPC value of 3021.8 mg GAE/100g and Golden Delicious (pulp) showed the highest TPC value of 1922.1 mg GAE/100g. **Manzoor *et al*. (2012)** determined the TPC of five varieties of apples i.e. Kala Kulu, Red Delicious, Sky Spur, Kashmiri Amri and Golden Delicious. Their study explained that out of these varieties, 80% methanolic extract of Kala Kulu and Red Delicious showed the highest TPC value i.e. Red Delicious peel (2587.9 ± 50.6 mg GAE/100g) and pulp (1475.5 ± 29.9 mg GAE/100g) while Kala Kulu peel (2274.8 ± 49.4 mg GAE/100g) and pulp (1388.4 ± 26.1 mg GAE/100g). These results showed that TPC previous studies values were higher than our current study. **Tareen *et al*. (2021)** determined that 80%

aqueous methanol extract of Red Delicious (peel) showed a TFC value of 2484.6 mg CE/100g and of pulp 1242.5 mg CE/100g. In our current study, aqueous extract of both Gacha and Kala Kulu showed lower TFC values as compared to the previous study. **Manzoor *et al.* (2012)** reported the TFC of 80% methanolic extract of Kala Kulu apple Peel as 1694.6 ± 37.2 mg CE/100g and in Pulp as 999.3 ± 17.7 mg CE/100g. These values are much higher than the TFC value of our study in which an aqueous extract of Kala Kulu was used 82.37±7.80 mg CE/100g.

Regarding antioxidant activity, **Saeed *et al.* (2023)** compared the methanolic and aqueous extracts of the apple peel powder for antioxidant efficacies. Their results revealed that methanolic extract showed a higher value, ranging from 25.40% to 69.2%, than aqueous extract which ranged from 14.30% to 45.62%. In our study, the aqueous extract of the Gacha apple showed 67.87% and the Kala Kulu apple showed 77.10% DPPH radical scavenging activity which was higher than the previous study. **Siddique *et al.* (2016)** studied two varieties of apples i.e. Red Delicious and Golden Delicious in their ripe and unripe form for alpha-amylase inhibition. Red Delicious ripe and unripe forms showed 79.50% and 70.2% inhibition respectively. These values were less than the percentage inhibition by the Gacha apple i.e. 86.69% but more than that by Kala Kulu apple i.e. 69.27% as shown in our study. The percentage of enzyme inhibition for ripe and unripe Golden Delicious was 77.33% and 67.6% respectively. These values are less than the percentage inhibition of the Gacha apple but the %inhibition value of Kala Kulu apple was in between the ripe and unripe Golden Delicious.

Antimicrobial activity assay of aqueous extract of two varieties (Gacha and Kala Kulu) of *Malus domestica* was shown in Figure 1(a, b) and Table 1. **Zhao *et al.* (2019)** experimented on the peel and pomace of apple (Golden variety) to determine their antimicrobial activity against *E. coli and S. aureus*. Their study finding showed that aqueous extract of apple peel and pomace had great antimicrobial activity. In a previous study, the zone of inhibition of apple against *Staphylococcus aureus* was 19.25mm (peel) and 12.75mm (pomace) which are more than our current study i.e. 10mm (Kala Kulu) and 11mm (Gacha). In a previous study, the zone of inhibition of apple against *E. coli* was 15.00mm (peel) and 11.50mm (pulp) while in our study, the zone of inhibition of Kala Kulu apple against *E. coli* was 13mm and the Gacha variety show no zone of inhibition. **Malaviya and Mishra, (2011)** have reported in their study that there was no zone of inhibition of aqueous extract of apple against *E. coli* similar to our study. Gacha apple did not show any zone of inhibition against *E. coli* but Kala Kulu apple has shown a 13mm inhibition zone.

Odibo *et al.* (2020) studied the anti-sickling properties of *Malus domestica* (Apple) in which results showed that apples protect the red blood cells from breakdown (hemolysis) by making them anti-sickle. Aqueous extract of apple inhibits red blood cells to become sickled and hemolyzed by 99.33%. It means only 0.67% hemolysis occurred, this percentage was much less than the percentage hemolysis value of the current

study i.e. 2.449% for Gacha and 5.122% for Kala Kulu apple.

FTIR Spectrum revealed that bands at 3647.2 show alcohols and phenols and bands at 2931.6 shows alkanes and carboxylic acids. **Roy *et al.* (2014)** observed -C=O- bond, in-plane N-H bending and C-N stretch at 1672cm^{-1} and in our studies, the bond type was the same as the previous study at peak 1653.1cm^{-1} and 1636.3cm^{-1}. Amide linkage generates the band at 1479 cm^{-1} while there is no peak at this point in the current study. A peak close to 2144cm^{-1} shows C=C stretching in the studies of **Roy *et al.* (2014)** while there is no peak shown in our study. The peak close to 3588 cm^{-1} shows intermolecular hydrogen linked networks of the -OH or -NH group and in our study same functional group was observed near this point at 3647.2cm^{-1} and 3278.2cm^{-1}. A band at 785cm^{-1} showed the presence of aromatic rings similar to the peak observed in our recent study. **Qadri *et al.* (2022)** observed the hydroxyl group's stretching at 3243 cm^{-1} which is similar to the peak present in our study at 3281.9cm^{-}A noticeable peak is obtained at 2991cm^{-1} due to N-H stretching while there is no peak at this point in the current study. In the 1500–1585cm^{-1} range, C=O stretching of carboxylic acids and esters was present while no peak was observed in this range in the recent study. Stretching of the C-O bond of sugar and organic acids shows a peak in the 1060–1170 cm^{-1} range that is similar to the current study.

CONCLUSION

The study was planned to compare the antioxidant, antidiabetic, antimicrobial, and cytotoxic potentials of two local varieties, Gacha and Kala Kulu, of *Malus domestica*. Structural characterization was done through FTIR analysis. Both varieties of *Malus domestica* showed great antioxidant potential. The Gacha apple has a higher value of TPC and TFC as compared to the Kala Kulu apple but in the case of DPPH, Kala Kulu showed greater values. Both varieties showed comparable alpha amylase values and lower values of hemolysis. Gacha apple showed greater antimicrobial activity against *S. aureus* as compared to Kala Kulu apple while no activity against *E. coli*. Both varieties exhibited diverse functional groups through FTIR. Overall, this comprehensive analysis sheds light on the multifaceted therapeutic potentials of the local apple varieties, emphasizing the need for further research to explore their bioactive compounds and mechanisms of action for potential pharmaceutical and nutraceutical applications.

REFERENCES

ALI, T., HUSSAIN, F., NAEEM, M., KHAN, A., AL-HARRASI, A. 2022. Nanotechnology approach for exploring the enhanced bioactivities and biochemical characterization of freshly prepared *Nigella sativa* L. nanosuspensions and their phytochemical profile. *Frontiers in Bioengineering and Biotechnology*, 10: 1-14. http://dx.doi: 10.3389/fbioe.2022.888177

CHAKRABORTY, A., DEB, J.S., SAHA, M., CHATTERJEE, S. 2023. Apple seeds: phytochemistry, medicinal property and toxicology. *International Journal of Pharmaceutical Science and Research*, 14: 1038-1045. k: http://dx.doi.org/10.13040/IJPSR.0975-8232.14(3).1038-45

HUSSAIN, F., AKRAM, A., HAFEEZ, J., SHAHID, M. 2021. Biofunctional characterization of red, black and white ginseng (*Panax ginseng Meyer)* root extracts. *Revista Mexicana de Ingeniería Química*, 20: 173-184. https://doi.org/10.24275/rmiq/Bio1735

JABEEN, R., ALI, T., NAEEM, M., HUSSAIN, F., DANISH, S., ALAHMADI, T.A., SAMDANI, M.S., ANSARI, M.J. 2023. An insight into biochemical characterization and explorations of antioxidant, antibacterial, cytotoxic, and antidiabetic activities by *Trachyspermum ammi* nanosuspensions. *Frontiers in Bioscience. (Landmark Ed)*, 28(12): 340. https://doi.org/10.31083/j.fbl2812340.

KAPPA, K., KALDERB, K., KIKASC, A., UNIVERC, T., PUSSAD, T., RAALB, A. 2023. Polyphenolic compounds in apple (*Malus domestica* Borkh.) cultivars are grown in Estonia. *Proceedings of the Estonian Academy of Sciences*, 72: 154-166. https://doi.org/10.3176/proc.2023.2.06

MALAVIYA, A., MISHRA, N. 2011. Antimicrobial activity of tropical fruits. *Biological Forum –An International Journal*, 3(1): 1-4.

MANZOOR, M., ANWAR, F., SAARI, N., ASHRAF, M. 2012. Variations of antioxidant characteristics and mineral contents in pulp and peel of different apple (*Malus domestica* Borkh.) cultivars from Pakistan. *Molecule*, 17: 390-407. https://doi.org/10.3390/molecules17010390

NISAR, B., SULTAN, A., RUBAB, S. L. 2018. Comparison of medicinally important natural products versus synthetic drugs-a short commentary. *Natural Products Chemistry Research*, 6: 308.DOI: 10.4172/2329-6836.1000308

ODIBO, A. O., AKANIRO, I. R., UBAH, E. M. 2020. In vitro investigation of the antisickling properties of aqueous fruits extracts of *Citrus paradisi, Musa acuminata* and *Malus domestica*. GSC *Biological and Pharmaceutical Sciences*, 13(03): 203-209. https://doi.org/10.30574/gscbps.2020.13.3.0412

QADRI, T., NAIK, H. R., HUSSAIN, S. Z., AHAD, T., SHAFI, F., SHARMA, M. K. 2022. Comparative evaluation of apple juice concentrate and spray dried apple powder for nutritional, antioxidant and rheological behaviour. *Quality Assurance and Safety of Crops & Foods*, 14: 74-85. https://doi.org/10.15586/qas.v14i2.1037

RAHMAN, M. M., BIBI, S., RAHMAN, M. S., RAHMAN, F., ISLAM, F., KHAN, M. S., KAMAL, M. A. 2022. Natural therapeutics and nutraceuticals for lung diseases: traditional significance, phytochemistry, and pharmacology. *Biomedicine and Pharmacotherapy*, 150:113041. https://doi.org/10.1016/j.biopha.2022.113041

ROY, K., SARKAR, C. K., GHOSH, C. K. 2014. Green synthesis of silver nanoparticles using fruit extract of *Malus domestica* and study of its antimicrobial activity. *Digest Journal of Nanomaterials and Biostructures*, 9(3): 1137-1147.

SAEED, M. K., ZAHRA, N., SAEED, A., SYED, Q. 2023. Physicochemical characteristics, total phenolic content and free radical scavenging activity of apple (*Malus domestica*) peel powder: Physicochemical characteristics of apple peel powder. *Pakistan Biomedical Journal*, 6: 7-11. https://doi.org/10.54393/pbmj.v6i02.847

SIDDIQUE, A., SULEMAN, M., BUKHARI, I. H., KHAN, M. H., AUJLA, M. I. 2016. Anti-enzymatic potential of two different varieties of *Malus domestica*, (malus communis desf, ripe and unripe fruits) and their constituent's analysis by Gas Chromatography and Mass Spectrometry (GC-MS). *Journal of Bioresource Management*, 3(1): 4. https://doi.org/10.35691/JBM.6102.0042

TAREEN, A. K., PANEZAI, M. A., SAJJAD, A., ACHAKZAI, J. K., KAKAR, A. M., KHAN, N. Y. 2021. Analysis of antioxidant properties, toxicity and mineral composition of selected apple (*Malus domestica* borkh) varieties from Balochistan. *International Journal of Agriculture, Environment and Bioresearch*, 06(6): 21-44. https://doi.org/10.35410/IJAEB.2021.5680

ZHAO, B., JIANG, X., CAO, J. 2019. Antibacterial activity of aqueous extracts of *Malus domestica* against some bacterial pathogens. *Archives of Infectious Disease and Therapy*, 3(2): 1-7. https://doi.org/10.33140/AIDT.03.02.4